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COLLECTED PAPERS

1918

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Reprinted from THE ANATOMICAL RECORD,
Vol. 14, No. 1, January, 1918

A pedigreed strain of 'twin' Oxytricha. J. A. DAWSON, Osborn Zoological Laboratory, Yale University. (Introduced by L. L. WOODRUFF.)

There appeared, on July 27, 1917, in a six-day-old stock culture from a pedigreed race of Oxytricha, several pairs of apparently fused animals. These were isolated and from them pedigreed strains of double or 'twin' animals have been carried, thus far, for over one hundred generations—'twin' producing 'twin' in each generation.

The two cells forming a 'twin' are closely and firmly fused *dorsally*, though the anterior ends are very slightly separated. The 'twins' swim actively with a characteristic rapid rotation on their long axis. The normal single animal of the race from which the 'twin' strain was derived has the macronucleus in two portions, as is usual in the genus Oxytricha, but no demonstrable micronuclei. Cytological study of the 'twins' shows simply a doubling of the normal characteristics.

Reproduction of 'twins' is by transverse fission, and has given, in certain cases, as high as 100 per cent of 'twin' progeny for five generations. Occasionally a 'twin' separates into two single animals, which are normal in every respect, except that the anal ends, where separation occurs, retain for an hour or so pointed projections. 'Twins' which show a slightly more marked separation at the anterior end form, when transverse fission takes place, two normal single animals from the anterior end and one 'twin' from the posterior end. Normal single animals resulting from 'twins' continue to divide as usual. The division rate of the 'twin' strain is equal to, or slightly greater than, that of the race of normal animals from which it arose. The proportion of the animals remaining 'twins' seems to be increasing as the strain grows older. The 'twins' of the present generation are apparently identical in structure with those which originated the strain.

A morphological and physiological study of this 'twin' strain is in progress.

A PRELIMINARY REPORT ON SOME GENETIC EXPERIMENTS CONCERNING EVOLUTION

[Reprinted from THE AMERICAN NATURALIST, Vol. LII., January, 1918.]

A PRELIMINARY REPORT ON SOME GENETIC EXPERIMENTS CONCERNING EVOLUTION

RICHARD GOLDSCHMIDT

THE nature of the gene, the variability of factors and the effects of selection are favorite topics of recent discussion, which is well known to geneticists. The latest publications of Jennings and Castle will stir up anew the uncompromising parties and lead to new discussions. We think it advisable, therefore, to give a brief account of certain parts of a very large body of work on fundamental questions of evolution which we have carried on during the past nine years, with the collaboration of Dr. Seiler and Dr. Poppelbaum. Although some parts of the work have been finished for some years, we do not intend to publish a full account until all the details are worked out. But as certain results have already allowed us to form definite views in regard to some fundamental questions of evolution, we may present them, together with examples of the experiments in question.

The majority of the experimental work in regard to the fundamental problems of evolution has been done with domesticated animals and their mutations (rats, *Drosophila*) or with Protozoa, which present the complication of asexual reproduction. We have directed our attention to experimental analysis of such phenomena in nature, which must give basic information about evolution, and we have studied the following phenomena:

1. *The Geographic Variation of the Gypsy-moth.*—This well-defined species is spread over a great part of the globe. In different habitats, however, different races are found. How many of these exist can not be stated, but the number must be extraordinarily large, as we know but two localities where the same race is found. We have found all the races to be perfectly fertile with each other, with the exception of one combination which has never been successful. We have studied and are still studying the genetics of a large number of these races.

2. *The Melanism of the Nun-moth, Lymantria monacha.*—The nun is one of the moths which have developed melanic varieties within recent times; and these melanic varieties, which were extreme rarities not many decades ago, have almost supplanted the original white form. We have worked out the genetics of this case and shall publish the details when conditions permit. Some of the results were read before the German Zoological Society in 1911 but no abstract was published.

3. *The Genetics of Alpine Varieties, especially of Parasemia plantaginis and the Italian Races of Callimorpha dominula.*—This work has been broken off by the war, but some of the first results are available.

We shall begin with a few facts concerning the geographic variation of the gypsy-moth. We have here a form that is spread all over Europe, through Siberia into China, and all over Japan, infesting, furthermore, part of the Atlantic coast of the United States. We have studied races from different parts of Europe and Japan and the Massachusetts form and we have found different forms in comparatively near-lying regions. Thus the races from the Rhineland, Silesia and Hungary are dif-

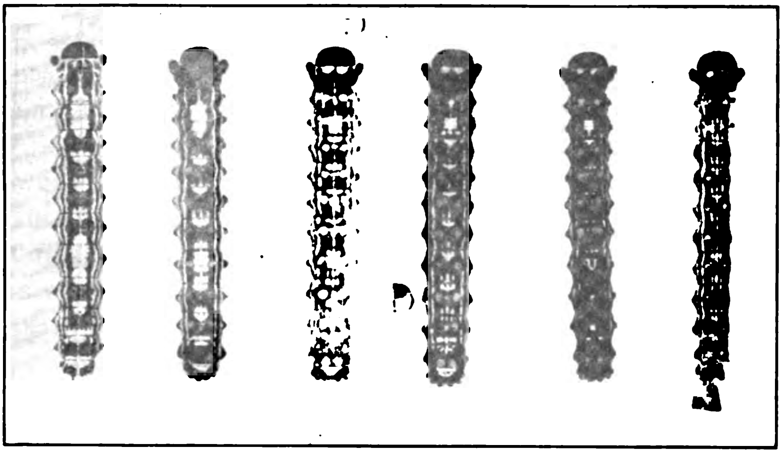

FIG. 1. Types of Caterpillars from different races after the first moult. Drawings by Mr. Yokoyama, Tokyo, 1914.

ferent from each other and from the Massachusetts race (probably imported from France). All of them are different from the Japanese races and these again differ in the different parts of Japan. The characters of difference are manifold; we shall confine ourselves here to a single character, more interesting and more characteristic than the others—the markings of the caterpillars. Fig. 1 shows caterpillars of a few races after the first moult. We see here some of the transitional stages from a very light to an almost black caterpillar. The genetic study of this character of marking shows that we are dealing here with a primary type of marking which belongs to the entire group of moths in a similar form, that is, the light pattern. All the darker forms have the same genetic basis of marking, on which, however, dark pigment encroaches increasingly until the markings practically disappear. We may now divide this increasing melanism into ten classes and place the lightest individuals in Class X and the ones without marking in Class I. It must be added that the dark series extends beyond Class I, but the difficulty of classifying them is such that no darker classes have been adopted.

The young caterpillars of the different races show markings which fluctuate around a mean at a certain point of the series and this behavior is remarkably constant for the different races. The following Table I gives a few polygons for different European and Japanese races.

TABLE I

CLASS FREQUENCIES IN PER CENT.

Breed	Race	I	II	III	IV	V	VI	VII	VIII	IX	X
WA3	H	..	..	..	..	..	..	..	38.6	54.5	6.9
UA24	K	..	..	..	..	..	..	15	64.7	20.3	..
UA17	F	..	..	..	..	..	1.5	60	38.5	..	..
VA2	O	..	..	..	..	..	8.2	48.8	31.4	11.6	..
VA8	G	..	..	..	..	..	..	11.8	57	31.2	..
WA4	A	..	..	..	..	19	43	38	..	..	..
WA1a......	S+	..	5.4	19.1	44	30.2	1.3	..	..	..	..
WA56......	M, S	100			..	..	..	..	..	..	..

In crosses of these different types F_1 is about intermediate, as some curves in Table II show.[1]

TABLE II

F_1. CLASS FREQUENCIES IN PER CENT.

Breed	Cross	1	II	III	IV	V	VI	VII	VIII	IX	X
VA17......	S × K	..	..	..	..	17.3	29.6	39.9	13.2	..	..
VA18......	K × S	..	..	..	9.2	33.3	14.4	42.2	0.9	..	..
VA21......	S × A	..	..	..	..	45.4	28.7	25.9	..	..	..
VA22......	A × S	9	20	29	11	15	13	3	..	..	..
VA23......	S × O	..	..	..	4.7	67.4	27.9	..	..	..	..
VA24......	O × S	..	..	..	..	9.9	66.9	22.4	0.8	..	..
VA37......	K × H	..	..	..	..	..	0.7	..	49.6	49	0.7
WA83......	G × M	..	..	2	24	34	32	8	..	..	..
WA88......	M × A	..	..	..	10	70	20	..	..	..	..

And F_2 gives a 1:2:1 ratio, or 3 light + medium : 1 dark, whatever races are involved. (This statement should be taken only on its face value. As a matter of fact, we find here, within the invariably present ratio of 3:1, very strange details of the kind described as "gametic contamination," and, furthermore, an obscuring of the ratio in earlier stages followed later by the right ratio, apparent lack of segregation, etc. From a purely genetic point of view, the analysis of these phenomena constitutes the most interesting part of this work, but it has no special relation to the problems here under discussion.) Back crosses, however, give a 1:1 ratio. The following table gives a few data of this kind.

TABLE III

Breed	F_2 From	Dark, Per Cent.	Light + Medium, Per Cent.
WA12...........	S×K	23.8	76.2
WA13a..........	K×S	25.5	74.5
WA24b..........	S×Ky	25.9	74.1
WA28a..........	A×S	25.3	74.7
WA31...........	O×S	26.2	73.8
WA18...........	S×H	30.2	69.8
ZA20............	O×M	22.1	77.9
ZA9.............	M×H	25.8	74.2
ZA12............	G×M	25	75

---

[1] We give here a few random examples. The amount of actual material is very large, as more than 100,000 caterpillars have been bred and studied. We refrain, furthermore, from all such details as behavior in reciprocal crosses, etc.

The actual curves look like the example in Table IV of an F_2 cross.

TABLE IV

ZA9 F_2 FROM M × H

	I	II	III	IV	V	VI	VII	VIII	IX	X
Individuals	24	..	..	..	13	9	29	15	3	..
Per cent	25.8	..	..	..	14	9.7	31.2	16.1	3.2	..

The sum of all the hundreds of curves shows that we are dealing here with a case of multiple allelomorphism: The pigment factor, producing the gradual covering of the markings, is present in the different races in different degrees, all being allelomorphic to each other.

Thus far we have dealt only with the very young caterpillars. Their further history in regard to the effect of these factors leads us one important step further. We find mainly the following types of behavior within the pure races: (1) Light marked caterpillars, which remain practically the same throughout the entire larval life. (2) Light-marked caterpillars which grow darker with every moult and finally are about medium or more than medium dark. (3) Light-marked caterpillars which change during the larval stage, so that they finally are all dark. (4) Medium light caterpillars of different degrees changing to dark during larval life. In the following tables we give a few examples of these races, showing the shifting of the type of marking during the stages of larval life. The large range of variation after the third and fourth moult visible in these tables is due more to a different speed of shifting in different individuals than to the initial variability. This is shown in Table VI, which gives an example of the shifting of types of pigmentation during the larval stages for a series of isolated individuals of some of the pure races.

The genetic analysis of this phenomenon seems to reveal the real nature of the multiple allelomorphs, which cause these different types of pigmentation and their behavior during development of the caterpillars. With-

TABLE V

EXAMPLES OF SHIFTING RACES

Race H

Class Frequencies in Per Cent.

Stage of Caterpillars	I	II	III	IV	V	VI	VII	VIII	IX	X
3	..	..	..	..	..	..	..	38.6	54.5	6.9
4	..	..	..	..	..	11.8		55.3	31.7	1.2
5	29.3	24.4	9.7	12.2	9.8	12.2	.	2.4	..	..
6	64	16	16	4	..	..	..	..	..	..

Race A

Stage of Caterpillars	I	II	III	IV	V	VI	VII	VIII	IX	X
3	..	..	..	..	19	43	38	..	..	..
4	..	..	..	..	35.5	58.1	6.4	..	..	..
5	14.3	42.9	4.7	9.5	28.6	..	..	..	..	..
6	100			..	..	..	..	..	..	..

Race G

Stage of Caterpillars	I	II	III	IV	V	VI	VII	VIII	IX	X
3	..	..	..	..	..	..	11.8	57	31.2	..
4	..	..	..	..	1.4	35.2	33.8	28.2	1.4	..
5	3.6	7.2	14.4	18	25	22.8	9	..	..	..
6	100			..	..	..	..	..	..	..

TABLE VI

EXAMPLES OF SHIFTING AS OBSERVED IN INDIVIDUALS WITH DIFFERENT NUMBER OF MOULTS

Race	Individual	Class of the Individual after Moult					
		2	3	4	5	6	Sex
H.....	ZA3.6	IX	VII	II			♂
".....	ZA3.7	VIII	VII	I			♂
".....	ZA3.4	IX	III	I			♀
".....	ZA3.1	VIII	VII	I	I		♀
A.....	ZA4.1	VI	V	III			♂
".....	ZA4.3	V	III	I			♂
".....	ZA4.4	VI	VI	IV	I		♀
".....	ZA4.8	VII	V	III	II		♀
".....	ZA4.13	VI	V	IV	I		♀
G.....	ZA6.11	VIII	VIII	VI	II		♂
".....	ZA6.7	VIII	VI	VI	IV		♂
".....	ZA6.5	VIII	VIII	VI	III		♀
".....	ZA6.6	VIII	VIII	IV	III	I	♀
".....	ZA6.17	VIII	VI	V	III	I	♀

out going into details, which would necessitate a multitude of tables and curves being given, the following points are of importance: (1) F_1 between a non-shifting light race and an always dark race is intermediate, or somewhat lighter in the beginning. But by progressive stages the hybrid caterpillars shift over into the dark classes.

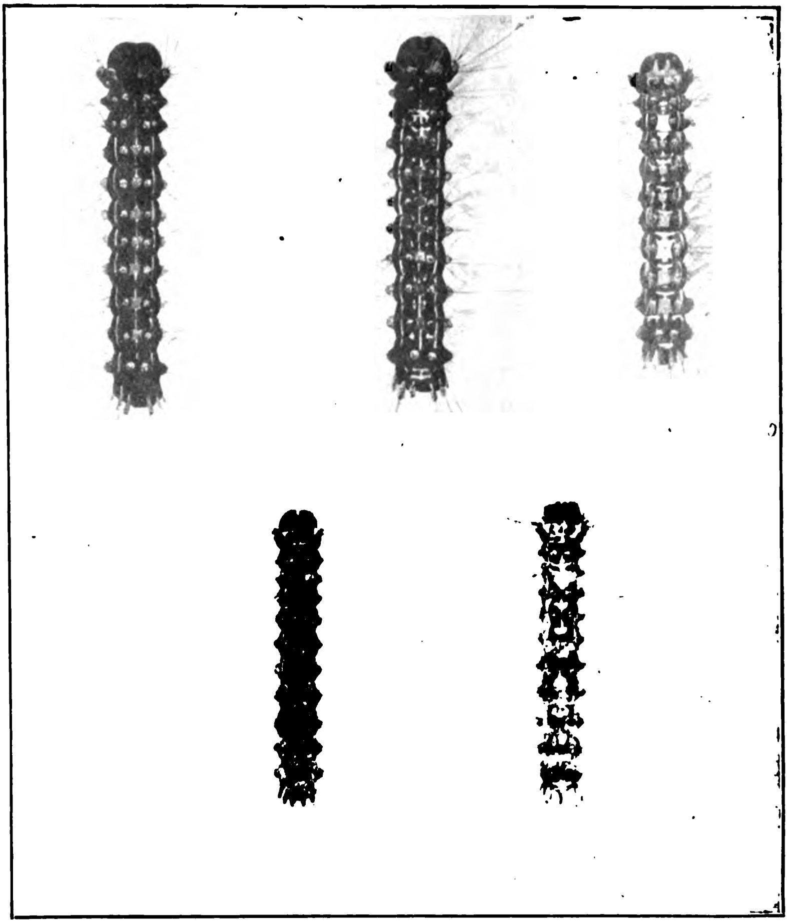

FIG. 2. First row: hybrid between the two parental races after second moult. Second row: Hybrid (left) and light parental race (right) after fourth moult. Drawings by Dr. Poppelbaum, 1912.

Fig. 2 represents caterpillars from a cross of this type. (The exact curves that belong with these pictures are reproduced in our "Einführung in die Vererbungswissenschaft," 2d edition, 1913, p. 170, Fig. 66, as an example of change in dominance during development.) (2) The same thing happens in certain F_2 crosses, reversing completely during larval life the original ratio of lights and darks. (3) The different races involved are characterized by a difference in the speed of differentiation, as shown in the actual curves. This velocity is also caused by genetic factors. Where these recombine with the pigmentation factors, the entire situation of the F_2 curve is shifted (without changing the 3:1 ratio), showing that the visible effect of the pigmentation factors is bound to a certain velocity of differentiation. (4) The shifting of the type of pigmentation from light to dark during larval life of certain races or hybrids is a process which progresses constantly with time. This is seen when isolated individuals are studied which belong to races that differ in regard to the number of moults and exhibit the shifting simultaneously. There are races where all the male caterpillars have four moults and the females either four or five; other races where the males have four, the females five; others where both sexes have five moults; and in the last case even a sixth moult occasionally occurs. In these cases we see that every new moult produces a further shift to the dark side of the curve, showing that the class of pigmentation to which a full-grown caterpillar belongs is in this case a function of the time of differentiation. The same fact can be demonstrated in a shifting race by prolonging the time between two moults by starvation (which succeeds only to a certain extent). In experiments of this sort it has been possible to get the shifted type of pigmentation, characteristic of the fourth stage, in some individuals in the third stage. Table VI also contains a few random data on the first point. (6) In shifting hybrid cultures there appear comparatively often mosaic-caterpillars, showing different classes of marking on right and left sides. The distance between these two different classes is approximately kept up when shifting occurs during development. The following example demonstrates this fact:

TABLE VII

	Class After Second to Fifth Moult							
	2		3		4		5	
	Right	Left	Right	Left	Right	Left	Right	Left
WA51, 27	IV	III	IV	II	III	I	I	I

These caterpillars always give normal moths and normal offspring.

A careful consideration of these points shows clearly what these multiple allelomorphs for pigmentation really are: They are different quantities of the substance which we call a gene which act according to the mass-law of chemical reactions, *i. e.,* produce a reaction or accelerate it to a velocity in proportion to their quantity. In our special case it means that the factor stands for a metabolic activity proceeding with a definite velocity dependent upon the quantity of the factorial substance present. This activity finds its visible expression in the deposition in the skin of increasing quantities of certain products of protein decomposition which as chromogens are oxidized into melanin pigments. The effect of the different quantities of active substance (enzyme?) which we call the multiple allelomorphs upon the progressive pigmentation

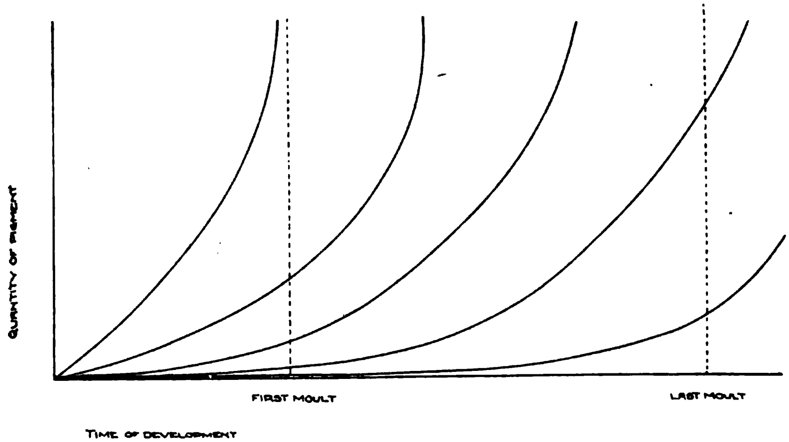

FIG. 3.

of the caterpillars is then represented by the graph on p. 36 (Fig. 3).

Given a definite quantity of the factorial substance and identical conditions, the velocity of the reaction is constant. Thus the final result depends upon the amount of the factorial substance present and the independently inherited rapidity of differentiation, which determines the situation of the growth-stages on the abscissa (the dotted lines). Thus the above quoted facts as well as the multitude of details not mentioned can be easily derived from this graph. The last named mosaics are of course the expression of small differences in the velocity of differentiation in symmetric halves of the body, which are well known to embryologists.

These conclusions in regard to the real character of multiple allelomorphs are the same as those derived from other characters in the same objects. In our work on intersexuality we were able to prove, to as great an extent as a genetic proof can possibly be carried, that the different geographic races of the same moth differ in regard to the absolute and relative quantities of the substances, which we call the sex-factors. In the genetic language of the present day we should call them, therefore, multiple sex-allelomorphs, a conception which indeed we have always used (without this recent term) since our first report about this work in 1911. In the case of intersexuality we can furnish facts very similar to those about the caterpillars, if we consider certain features of the wing colors. In normal males a certain amount of pigment covers the entire wing, whereas the female wing is unpigmented. This pigment is formed by the oxidation of a chromogen deposited within the scales. There it flows from the wing veins with the blood. By a detailed analysis we are able to show that an intersexual male is a genetic male which developed as such up to a certain point when the development suddenly began to continue under the aspects of femaleness. One of the results of male metabolism is the production of these chromogens in late larval stages. This production is therefore stopped when female metabolism sets in; when then the time arrives in development, when the chromogen spreads over the wing scales, its available amount is proportional to the relative lateness of the reversal of sex. Therefore, with increasing intersexuality, the pigment flowing from the veins covers a smaller and smaller area of the wing, finally being confined to the neighborhood of the veins. As[2] the analysis of the other intersexual organs allows an accurate determination of the time factor involved, we have here a very close physiological parallel to the facts about the caterpillars.

In most other cases of multiple allelomorphism only the results can be seen, and it will be difficult to work out the time factor, which proves that the multiple allelomorphs are different quantities of an active substance. (Some botanical subjects ought, however, to be favorable.) But in comparing the other facts about multiple allelomorphs with our cases, we feel confident that, whereever a similar analysis can be applied, the results will be the same. For example, all the cases of quantitatively different pigmentation, which are of multiple allelomorphic nature, like Castle's hooded rats or our different cases of melanism in moths, show that the effect of the different factors is that different quantities of pigment spread from different "points of outlet," which of course are hereditary traits of the species or group; the similar effect, therefore, leads to suspect a similar cause.

If our conclusions regarding the nature of multiple allelomorphs are accepted, it must lead to a different intellectual attitude toward the problem of variability of genes, which is so important for evolution. The opposition to the view has been, we believe, primarily on aprioristic grounds. In the long controversies of recent years regarding the interpretation of Castle's work the logical side of the case seems to have always been in the foreground. The same is the case when E. Baur calls our views in regard to the variability of the sex-factors *a priori* inadmissible. We believe that this intellectual attitude toward the problem is the result of Johannsen's doctrine of agnosticism in regard to the nature of the gene, which resulted in a kind of mystic reverence, abhorring the idea of earthly attributes for a gene. (Our distinguished opponents will excuse this somewhat extreme statement.) If, however, it can be proven that genes are substances with the attribute of definite mass, it would be illogical to deny their variability. Nobody will claim that a gene is a substance that passes unaltered from generation to generation. The elementary facts of development and regeneration show that this substance grows, at least, and increases in quantity. If, now, the substantial basis of heredity in the sex-cells is established by the assembling of all the factor-substances in their characteristic quality *and their correct quantity,* the situation is the same for the gene as for any other organic process: the varying conditions of the surroundings of the gene cause a certain amount of fluctuation in its quantity. This conclusion entirely changes the logical aspect of the question, whether or not a change of the gene by selection of variants is possible.

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[2] See pictures in *Jour. Exp. Zool.*, 22, 1917, pp. 614–15.

The strongest point of the anti-selectionists was that it is absurd to assume that a selection of somatic fluctuation has anything to do with the characters of the germ-plasm. With the quantitative view, however, which we believe to have proven in two elaborate cases, this situation changes. The somatic character in question, say amount of pigmentation, can only change toward a plus or minus side. This change is caused directly by a difference in the velocity of the reaction of some metabolic process which results in the deposition of pigment. Such a change of velocity of reaction, however, can be produced either by the action of the medium, and then it is a modification, or by fluctuation in the quantity of the gene, causing increase or decrease in the velocity. The resulting variation is of course, phenotypically, the same. Selection, therefore, may be ineffective, if a modification only is selected; it will be partly successful if a combination of plus-quantity with plus-modification is selected; and fully successful if the exclusive result of plus-quantity of the gene is selected. The *deus ex machina* modifying factor, which, moreover, does not fit the decisive genetic facts in the most discussed case of Castle's rats nor our cases, thus becomes superfluous.

It is, moreover, perfectly logical to assume that selection of either plus or minus quantities of the genes changes the mode of the fluctuation of this quantity correspondingly in the succeeding generation. If the different quantities of the substances, which constitute the systems of multiple allelomorphs, are inherited, then every other quantity is also inherited. If the presence of the quantity p in the germ cells of the parents causes the reappearance of the quantity p in the germ cells of the children, the same fact applies to the quantities q, r, s—to every quantity which is present or has been selected. Selection can, therefore, change the quantity of the gene, and also, therefore, the somatic characters caused by quantitative differences in the gene, until the physiological limit is reached. This limit may be the limit for the character in question—for example, no pigment, self-color—or it may be the limit set by the necessary coordination of developmental processes. For example, in the development of a moth a certain gene causes, at a certain moment—during pupation—the evagination of the imaginal disks of the antenna. The correct quantity of the gene causes this process to take place at the correct time. A quantitative variation of the gene would cause the evagination to take place at the wrong time. We have, indeed, had strains of caterpillars where in many individuals this process took place in the last stage of the caterpillar, giving caterpillars with pupal antennæ. The quantity of the gene in question was in these cases not coordinated with the other genes and the action was produced too early. It is evident that quantitative changes of this kind will lead to physiological impossibilities, monsters, etc. Here, then, is again the limit for selection of factorial quantities. It need hardly be added that such selection is necessarily orthogenetic.

Our own experiments in this line are, as far as they go at present, in perfect accord with Castle's work. We have, moreover, applied another type of experimental test, namely, selection in F_1. If a given pair of multiple allelomorphs differs in regard to the quantity of the factorial substance and this quantity is subject to fluctuation around a mean, the variability of the character in F_1 is caused by the usual agencies producing fluctuations as well as by the different combinations of the parental quantitative values. Selection in F_1 ought, therefore, to influence the curve in F_2 in a certain number of cases, namely, when the plus or minus individuals are genetically plus or minus. Within the normal segregation of light and dark individuals in the 3:1 ratio a shifting of the mean for lightness and darkness must take place. In a series of such experiments we had a number of positive results. The following Table VIII may serve as an example:

TABLE VIII

F_2 with Selection in F_1 from Cross K × S

	In Third Stage									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Plus selection	25.5	. .	. .	7.3	12.7	9.1	30	15.4	. .	. .
Minus selection.	25	5.7	26.9	15.5	17.2	3.2	6.5	. .	. .	. .

We believe that these facts and interpretations have a definite bearing on the problem of evolution. The first step in the differentiation of species which occurs in nature seems to be the formation of geographic races. The entire bulk of modern evidence in ecology tends to show the existence of clearly defined local forms for very restricted areas. For example, the ichthyologists differentiate forms of Salmonids and Coregonids for practically every river and lake; in the same way in the lower organisms, like Daphnids and Rotatoria, different forms appear in different regions. The ornithologists describe different races for every river basin of the affluents of the Amazonas; the mammalogists do exactly the same thing for every area which was thoroughly covered. Where breeding experiments have been carried on it has been shown that the geographic races may be perfectly fertile with each other and may produce fertile offspring. In some cases, however, the transitional stages toward sterility are found. Thus the production of intersexual moths in crossing geographic races can be regarded as a step toward increasing incompatibility, which in one of the crosses attempted by us was an absolute one. In other cases only a small percentage of the offspring of the hybrids could be reared, as in the crosses of the North and South European *Callimorpha dominula.* We, therefore, with many evolutionists, feel convinced that the geographic races are the most important visible steps in species-formation in nature.

If we now look into the characters distinguishing geographic races, we very often find certain qualitative differences most conspicuous, for example, exchange of red and yellow color in the moths. A close study of definite examples, however, reveals that these differences are often more conspicuous than important. This is shown by the only group of information in the animal kingdom which we have both by ecological and genetic work—the geographic variation of land snails. The facts about the extreme variability of *Helix, Achatinella, Partula,* etc., are well known, as well as the irregularities in the confinement of definite types to definite localities. We have been so fortunate as to gain some insight into these facts through a very interesting collection which Dr. Haniel made in Timor and studied under our direction (not yet published). It was evident here, as in the other cases, that a series of unit factors for number, color, form of bands and ground color, which recombined freely, was involved. And practically all the combinations could be reduced to the genetic factors which Lang worked out for *Helix*. But, exactly as in the classic cases, there was no possibility of stating a definite relation of these factors to the grouping according to localities. In some localities certain factors or combinations did not occur, but the attempt to classify the material along this line proved a failure. However, every group from each locality exhibited beside these factorial recombinations certain quantitative characteristics of size, proportions, etc., of the shell which were characteristic for definite localities. These, however, are the characters which probably fall in line with those caused by the quantity of the genes.

The difficulties which the facts of geographic variation create for the conception of species-formation by selection have often been discussed. Bateson in particular ("Problems of Genetics") scrutinizes them from the modern genetic point of view. They are indeed insuperable if all characters which show variations and recombinations are considered from this point of view. The extreme irregularity, for example, of the local combinations of types of shells in *Helix, Partula* and *Achatinella* makes it impossible to regard them as local adaptations. This is certainly true, but may be without any bearing on the species question at all. The factors and recombinations occurring in *Helix, Achatinella* and *Prodromus* are more or less the same, just as are the recombinations of coat colors in different rodents. They constitute a set of mutations and their recombinations which are proper to the type of germ-plasm of the group. They occur, recombine or fail to appear as chance wills, and seem to have no special selective value. We do not think that these are the characters which play a part in the evolution of species; they are, in most cases, independent of adaptation.

There are, however, reasons for supposing that such differences of characters as are based on the quantitative differences of the gene are those which are influenced by selection and are important for the formation of the first steps toward diversification of species. We base this opinion on the following facts:

One of the few cases where selection in nature has apparently been seen at work under our eyes is the much-quoted case of melanic moths. We started in 1908 to work out the case of the nun, *Lymantria monacha.* The dark varieties of this moth have been known as rare occurrences for over a century. But only during the last decades have they spread and almost replaced the white forms. The analysis of the genetics of this case shows that the dark form is a dominant mutation to the white and that the many different stages of darkness, which form a complete series from white to black, are produced by sex-linked multiple allelomorphs. (Unfortunately, the interesting details can not be given at present.) How is it, now, that these combinations have come to replace the original form? Many hypotheses, some of them very strange, have been put forward; but it seems to us that the case is comparatively simple. The dark forms are stronger, more lively, better fliers, as far as we can tell from our experience with the animals in captivity. They are also larger (see Fig. 102, p. 267, in our "Einführung in die Vererbungswissenschaft," 2d ed., 1913). The melanism is in this case, therefore, only the most conspicuous superficial feature of a quantitative and progressive change in a gene which causes a definite metabolic condition, resulting in hardiness as well as in the deposition of more pigment in the wings. The quantitative change has here a superficial expression and is therefore easily recognizable. But this visible pigmentation is not the really important character. How is it, then, that these melanic forms, and other forms in similar manner, have established themselves so suddenly? We may venture to point to the facts that the selection, as has often been stated, has occurred especially near the larger cities, and that the period during which this selection has taken place is the period of industrial development, *i. e.,* of restriction of forested areas near the cities. It is, furthermore, the period of scientific and intense forestry and of economic entomology. Here we have the probable agencies that made life difficult for the moth and gave a great selective value to that advance in hardiness which lies behind the melanic appearance.

We should point out here the difficulties which arise in the criticism of definite views of evolution on the basis of facts not analyzed genetically. The selective value of a climatic character may often be doubted on the ground that the same type occurs in a very different area admixed with the local form. But genetic analysis may often show that what appears to be the same type is in reality a different thing. The north European Arctiid, *Callimorpha dominula,* has wings marked with red; the Italian form has wings marked with yellow. In certain localities (one of them near Berlin) a yellow sport of the red form regularly appears, apparently the same form as the Italian one. We, as well as others, have crossed these forms. The yellow sport is a simple recessive to red and segregation occurs in the 3:1 ratio. The Italian yellow form, however—at least the ones from the Abruzzi, which we used—crossed with the red northern form, produces intermediate orange in F_1 and in F_2 every shade from red to yellow. The two yellows, which look alike and probably are chemically alike, are nevertheless products of a different metabolic process. In the sport the same metabolic process which usually leads to red pigment is changed by mutation only to the extent of the color change in the end-product. In the southern form a different type of metabolism results in the formation of yellow pigment, and the cross is therefore an entirely different cross, with different results.[3] As a matter of fact, the latter cross shows very much diminished fertility, as Standfuss has already pointed out. This shows how unsafe the ground is on which criticism of evolutionary questions without genetic test is based. That our example is not an exception is proved by the fact that Standfuss long ago formulated the rule, that when two forms coexist in the same locality and are able to interbreed, they do not produce intermediates; but when the forms are geographically separated as local races, crosses between them result in a series of intermediates. Bateson says: "In this aphorism there is a good deal of truth." We think that the rule expresses the difference between a non-adaptational chance mutation and the adaptational change in the factorial quantities which may lead to a similar-looking, but physiologically different character. This character, although, like the non-adaptational one, is itself of no selective value, is the result of a general physiological change which does have a selective value.

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[3] We may point out that herefrom a rational interpretation of dominance and blending can be derived.

This will become still more evident if we return once more to the study of the gypsy-moth. In studying the relations of the different geographic races as characterized by the multiple-allelomorphic characters in question, we find that these characters are paralleled closely by differences in the life-cycles. Without going into details, we may state as a fairly general rule that the races with high degrees of pigmentation in the later stages are the ones which show a fast development, comparatively short larval life and a long period of hibernation. The light races have a comparatively long larval period and a correspondingly short period of hibernation. The former races, furthermore, inhabit the areas where a long and cold winter occurs, while the latter are endemic in places which have a hot summer, early spring and mild winter. One might think that these different characteristics were simply the direct effect of temperature conditions. But that this is not the case is shown by the constancy of the differences when the races are bred in a different climate and also by experiments on the physiology of hibernation, which have convinced us that the time relations of the life-cycle are—of course, within the limits of fluctuation—a heritable trait of rhythmic character. These facts show where the adaptational character of the differences of the geographic races lies: the adaptation which fits the different milieus is the life-cycle (in a broad sense). The visible distinctive characters of the races—aside from additional mutations of a non-selective nature—are nothing but the products of reaction of different types of metabolism, allied with the different time relations of the cycle. The method of the formation of geographic races in this case must, therefore, be the following. The first conquest of a new territory is of course only possible when the animal is preadapted, along general lines, to the new medium. But that it can maintain itself depends upon its power of special adaptation. The gypsy-moth, for example, has repeatedly been brought into England, but it has never established itself there. In the case of this form the special adaptation means the coincidence, in the first place, of the life-cycle with the seasonal cycle in nature. And it is here that all the discriminating effect of selection comes in. The quantitative changes of the genes which cause the time relations of the cycle are then the material for selection, and selection acts according to Darwinian principles until the equilibrium is established. Thus the genetic study of the quantitative changes of the gene reveals anew the truth of Darwin's conception. Furthermore, we see here how sterility of hybrids or complete incompatibility of new forms may arise. We have proved that the quantitative differences of the sex-factors, which are themselves nothing but adaptations to the time-relations of the cycle, are among the characteristic differences of these races.[4] There are, moreover, responsible for the incompatibility in regard to sex which results in intersexuality after crossing. Changes of exactly the same type may easily make any cross-breeding impossible, since no organism can develop unless all the processes of differentiation are coordinated in respect to their velocity. Here we see, finally, why geographical races are so often uniform and are characterized by certain traits of a quantitative character even when additional mutations and their recombinations make them at first sight appear diversified. This uniformity indicates the adaptational type produced by selection of the quantitative variations of some vital gene; the differences are only a difference in apparel.

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[4] Compare also Pflüger's and R. Hertwig's work with frogs and Cuénot's with starfish, demonstrating similar facts in regard to geographic variation of sexuality.

In conclusion, we may point out three groups of facts which, of the greatest importance for evolution, have always been a hard nut for the mutationists to crack. The first is the series of temperature-experiments in Lepidoptera—and similar experiments in Amphibia, Crustacea, etc.—that lead to the production of aberrant forms which resemble closely certain geographic varieties. But, with the exception of certain often-quoted cases, these aberrations are not hereditary. In the light of our experiments these facts are not surprising. The effect of the temperature experiments is to change the normal time-curve of certain metabolic processes. The effect is, therefore, due to this change of one of the variables of the reactions in question. The quantitative change of the substance of a gene, however, which we found to be at the basis of the geographical variations, also produces a difference in respect to the time-curve and therefore the same effect, this time a heritable effect. If we now select the plus individuals in this type of experiment—and this applies to all analogous experiments—we may simply select a modification. But we also may select the combination of a plus-modification with a plus quantity of the gene in question. If the experiment is repeated, the next generation will then show a still stronger reaction, or, if the experimental influence is not repeated, there will be an after effect of the experiment on the parents. It is remarkable that such results, which were to have proved the inheritance of acquired characters, always turned out, when characters relating generally to the life-cycle were in question, characters which also appear in the geographic races of the form. Extreme mutationists used to deny or disregard these facts. Here we have a simple explanation for them which both does justice to the facts themselves and falls in line with modern genetic views.

Furthermore, we now see the exact meaning of Darwin's view, which he had to express in a somewhat ambiguous way on account of the lack of experimental data which would have permitted clearer expression. His essay of 1842, the forerunner of the "Origin of Species," begins with the words: "An individual organism placed under new conditions sometimes varies in a small degree and in very trifling respects, such as stature, fatness, sometimes color, health, habits in animals and probably disposition. . . . Most of these slight variations tend to become hereditary." This statement shows clearly what Darwin had in mind. If he assumes that some variations, which are produced by change of conditions, are sometimes non-heritable, but tend to be inherited, we can now explain what this means. The variations which, as geographic races, form the first steps in the formation of new species are indeed exactly the same whether or not they are inherited. Their direct physiological cause is also identical, being a change in the rate of a definite process during differentiation. Only the ultimate cause is different; in the one case the original quantity of the gene determines the rate of differentiation—which then is hereditary—from the beginning; in the other case an outside factor is active, retards or accelerates the same reaction to the same degree. With this additional bit of interpretation, Darwin is right, after all.

The other group of facts includes certain details of mimicry (mimetism). We believe that the general principle of mimetism has been fully explained genetically by Punnett. But there are certain details which his selectionist opponents point out which constitute strong evidence against Punnett's view. We think that the most valid argument against the Mendelian view of mimetism has been derived from the facts about the parallel geographic variation of model and mimic. If our genetic conception of geographic variation is correct, this point is not difficult to understand. If the resemblance of model and mimic is based on the presence of similar chance- or non-chance combinations of genetic factors, and if geographic variation consists in the specific adaptation of the quantity of certain genes to a required velocity of some vital reaction, it is very natural that similar genes in model and mimic should be in exactly the same situation and should undergo parallel changes.

The third important set of facts to be considered is the problem of domestication. Darwin's view is well known, as well as the solution of a great part of the problem through Mendelism. The latter shows that selection of the recombinations after cross-breeding (besides picking of mutations) is the chief source of success in domestication. (See our demonstration of this fact regarding the improvement of pigs in "Einführung in die Vererbungswissenschaft," 2d ed., 1913, pp. 276–80.) That this fact was well known to Darwin is shown, for example, in his report about Lord Orford's greyhounds ("Variation of Animals," etc., Ch. 1). But he believed, in addition, in a positive effect of selection of small variations. Wherever he tabulates such characters, most or all of them are quantitative characters of a kind which we can assume to be dependent upon the presence of definite quantities of a gene. Here we may have the solution of the difficulties which the problem of domestication affords in spite of mutation and recombination. No doubt the high capacity for fattening was crossed into our hogs with Asiatic forms. But selection of plus-quantities of the responsible gene enabled us to obtain the character as it stands to-day.

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CONTRIBUTIONS TO THE STUDY OF CELL MECHANICS

II. MONASTER EGGS AND NARCOTIZED EGGS[1]

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TEN TEXT FIGURES AND FIVE PLATES

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INTRODUCTION

The present contribution embodies the results of a microscopic study of monaster eggs in sea urchins, together with some experiments made in an attempt to understand the meaning of the behavior of certain cell elements. In making an analysis of the observed facts, we have been brought face to face with so many mooted questions of cell mechanics, which have been left unsolved, that it is best, perhaps, to define the view-point from which the present research was undertaken. Cytologists seem to be generally agreed that, in the ultimate analysis, the problem of cell division is one for the colloidal chemist. On the other hand, the morphological phenomena, which are expressions of chemical and physical changes, are extremely varied in cells of different types, and any one familiar with the vast amount of cytological literature which has accumulated the last twenty years, must realize that scarcely any two authors are in agreement as to the really essential features with which the chemist must work. Simple morphological studies, while extending our conception of the cell in its varied activities, cannot bring us nearer to this goal. It is only by experimental studies, such as the Hertwigs, Boveri, Wilson, Conklin, Morgan, and others have given us that real progress has been made. These authors have shown that much light may be gained by subjecting one type of cell, such as an egg cell, to abnormal conditions. When this is done the forces, which in normal cleavage work synchronously, run their courses independently and the experimental cytologist may sift out the essential from the non-essential, and eventually formulate the questions which the physical chemist must solve, as soon as the nature and physical behavior of colloids are more thoroughly understood.

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[1] The present work was carried on at the Zoological Station at Naples, at the Woods Hole Marine Biological Laboratory, and at the Osborn Zoological Laboratory. Contribution No. 136 from the Zoological Laboratory, University of Texas.

The present study was undertaken with the hope that an analysis of monaster eggs would enable me to clear up some of the confusion which has existed in cytology. The first part of this work was undertaken at the suggestion of Theodor Boveri, at Naples, during the spring of 1914. Here the observation on the living eggs were made and material for cytological study preserved. The experiments with the narcotic were made at Woods Hole, Mass. While in Naples I enjoyed the privilege of Professor Boveri's experience and advice. The observations on the living eggs were known to him but the results of the cytological study together with the conclusions I have drawn from them, were not completed until after his death.

MONASTER EGGS

Although monaster eggs were known long before, Boveri seems to have been the first to realize the value of such eggs in analytical studies of cell dynamics and both he and his wife published papers dealing with the external behavior of the eggs (Th. Boveri, '03), and the behavior of chromosomes, centrosomes and other cell parts (M. Boveri, '03). Neither of these authors, however, had undertaken a study of the movements of the protoplasm which are such a conspicuous feature of monaster eggs; especial attention was therefore given to this in the present work.

A great variety of agents act upon the normal sea urchin eggs in such a way that only one division center appears when the division cycle is initiated. Numerous chemicals produce monasters, strychnine (Hertwigs, '87), $MgCl_2$ (Wilson, '01 a), carbon dioxide (Herbst, '14). Many narcotics do the same. Chloral hydrate (Hertwigs, '87), ether (Wilson, '01 b), phenyl urethane (Painter, '15). Mechanical stimuli act often in the same way. Thus Boveri found that by shaking eggs a few minutes after fertilization, a good percentage of monaster eggs often resulted.

The last named method was employed, as it was the simplest, and introduced no additional factor, such as the use of chemicals might have done. As soon as the fertilization membrane appeared, in eggs artificially fertilized, they were placed in a test tube, with as little water as possible and shaken violently by hand for twenty seconds. Precaution was taken to keep the warmth of the hands from increasing the temperature of the eggs, and after shaking they were placed in shallow dishes and allowed to develop.

The production of monasters is capricious and seems to depend as much upon the condition of the eggs themselves as on any other factor. If one treats two sets of eggs from different females in just the same way, shaking the same amount and at the same time, in one set one may get ten per cent monasters, and in the other only a few or none at all. Experiments were made to determine what was the best time to shake the eggs. The results of these different experiments are given in the tables below. From these it will be seen that when eggs were shaken between four and eight minutes after fertilization, the largest number of monasters were produced.

The extreme variability which monaster eggs show in their later behavior has been noted by Wilson ('01 a), Boveri ('03) and myself ('15). For the sake of clearness I have given the main paths of divergence, as I have found them, in the following scheme.

TABLE 1

Experiment of March 2

MINUTES AFTER FERTILIZATION SHAKEN	NUMBER OF NORMAL	NUMBER OF MONASTER	PERCENTAGE OF MONASTER
2	391	9	2.2
4	411	83	18.0
6	385	115	23.0
8	387	125	24.0
10	470	44	9.0
12	410	5	1.0

TABLE 2

Experiment of March 4

MINUTES AFTER FERTILIZATION SHAKEN	NUMBER OF NORMAL	NUMBER OF MONASTER	PERCENTAGE OF MONASTER
4	221	48	21.0
6	480	26	5.2
8	200	5	2.5
10	500	25	5.0

TABLE 3

Experiment of March 11

MINUTES AFTER FERTILIZATION SHAKEN	NUMBER OF NORMAL	NUMBER OF MONASTER	PERCENTAGE OF MONASTER
3	380	12	3.0
5	395	51	10.0
7	296	32	10.0
9	202	27	10.0
11	300	0	0

Monaster eggs may:

1. **Divide early without passing through a period of protoplasmic movement. Frequently spiral asters are found in such eggs.**
2. **Pass through division cycle and:—**
    - **(a) form amphiasters, divide and develop normally.**
    - **(b) form tetrasters or triasters.**
    - **(c) form multiple asters.**
    - **(d) form monasters.**

In the following account the description of the living egg will first be given and then the results of the study of sectioned material.

Changes in living egg

The monocentric condition is a normal phase in the development of an amphiaster, (Boveri '00, p. 155). Consequently, monaster eggs cannot be certainly distinguished until the majority of the controls are in some late phase of the first division cycle. By this time the single aster will have reached considerable size, and is seen approximately in the middle of the egg with radiations running in all directions. The size of the aster is variable.

As the control eggs divide, the monaster eggs show little change except a slow increase in the size of the clear centrosphere. As a few of the controls are entering the early phases of the second cleavage, some of the monaster eggs undergo changes which lead to the formation of an amphiaster, and such eggs divide. The vast majority of eggs, however, show no change until about half the controls are in the 4-cell stage. The single aster, which by this time is very large, begins to undergo a striking change. It slowly becomes flattened on one side, then concave, and finally saucer-shaped. As this is going on it moves through the egg's substance until it lies well against one side of the egg (fig. 1). The convex side of the aster goes forward, during the movement and the fibers on the concave side seem to reach nearly to the opposite side of the egg. There is a great amount of variability in the amount of the flattening, and of the retreat of the aster, as a glance at text figures B, C and D will show.

As the aster assumes its new position, with high power, one sees little clear vesicles lying on the edge of the centrosphere on the concave side. These are the chromosome vesicles which slowly fuse to form one nucleus, as the movement of the protoplasm, described below, sets in.

Following directly on these changes in the aster and in the chromosomes, we have a series of phenomena which involves the protoplasm of the egg. This is first seen in the ectoplasm (or 'Verbindungsmembran' of Herbst, '00), which, on the side of the egg opposite the concave side of the aster, (fig. 1) begins to swell or blister. While this layer remains thin and difficult to see, except in dim light, on other parts of the egg, in the region opposite the aster it increases in thickness and frequently becomes granular and more or less opaque.

After the membrane swells, the whole area of protoplasm, which it covers, begins to undergo a series of changes of contour during which little ridges and depressions appear. These rapidly change into pseudopod-like processes (fig. 2. In this drawing as in the rest of the figures on this plate, the ectoplasmic layer is not shown) and the whole surface of the egg becomes involved in a series of slow protoplasmic movements. The mobility of the protoplasm is extraordinary. Figures 2, 3, 4, 5, 11, 12, 14 show typical cases in various phases of the movement. The egg becomes flattened as the movement progresses and the finger-like processes undergo rapid changes in shape like the pseudopods of an amoeba, and these processes may even be cut off entirely from the egg (figs. 4 and 5). In this event, however, they continue to undergo changes in shape for a short time.

As the radiations of the aster diminish in intensity and disappear, the protoplasmic movements begin to subside. The finger-like processes are withdrawn; the little protoplasmic balls again fuse with the egg, (figs. 12 and 14) and the whole egg becomes rounded out, in most cases, before the next division cycle is well advanced. The large excentric nucleus and the swollen ectoplasmic layer are the only features which show that the egg has passed through the monaster cycle.

The protoplasmic streaming is extremely variable not only in the eggs of different females, but in the eggs of one individual. The typical course of events is shown in the camera and free-hand sketches reproduced in figures 1 to 5 and 11, 12, 14. But in some eggs the aster does not flatten so much as to become concave, nor does it move very far in the egg substance. Going hand in hand with this, the movement of the protoplasm is not so severe. A full discussion of such eggs will be given later.

One very striking phenomena which accompanies the protoplasmic movement, is the flow of the material from the cortical layers of the egg into the protoplasmic processes. This is best seen in heavily pigmented eggs, such as those of Arbacia, from which figure 11 was made. The same flow is seen in Strongylocentrotus, although it is not conspicuous because of the lack of pigment.

The time when the movement of the protoplasm is seen is extremely variable when measured in terms of the control eggs. It may be observed anywhere between the 2- and 8-cell stage. But in any single egg it only follows after certain definite changes in the aster and in the chromosomes. The length of time which the movement lasts is also variable. Sometimes the egg will be rounded up twenty minutes after the swelling of the membrane began, or it may be double that time before the period of movement is over.

The second division sets in, usually, just as the normal rounded form is being resumed (fig. 14) and the divergence which various eggs show in their behavior (see p. 449) begins.

In the large majority of cases, the second division cycle is accompanied by the formation of an amphiaster (figs. 12 and 14). Except for the excentric position of the spindle and the large asters, such eggs appear normal. When cytoplasmic cleavage sets in, however, we do not have the clean cutting through of the protoplasm. Instead, there is first a very pronounced swelling of the ectoplasmic layer in the cleavage plane, which amounts to a disintegration, often, on the side farthest away from the spindle (fig. A). This is followed by severe protoplasmic movements in the cleavage plane (figs. A_1, A_2

and figs. 7, 8) which eventually result in a separation of the two blastomeres, and frequently also, in the formation of a third (enucleated) ball, which may or may not fuse again with one of the blastomeres. We witness here again, during this division cycle, the formation of pseudopod-like processes (fig. 7) especially on the side farthest away from the spindle. Also, the two sister blastomeres become fully separated (fig. 8) when cleavage finally takes place.

Eventually the two blastomeres round up and the third division cycle sets in. The further history of these eggs has been described elsewhere (Painter, '15). Isolated eggs have given me some normal plutei. Boveri ('05) obtained the same results.

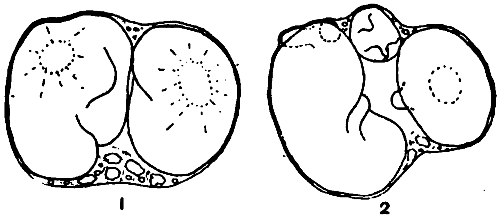


Fig. A

In a few monaster eggs, triasters and tetrasters are obtained at the second division cycle. As far as they were followed, they did not differ in their behavior, from dispermic eggs, and eggs isolated did not develop normally.

At the second division cycle the last two types of eggs mentioned on page 449, under c and d, can not be distinguished. One finds the reappearance of a single division center; this, however, is marked by the small size of the centrosphere, which rarely approaches, in my experience, the size of the monaster in the first division cycle. After a variable length of time, movements of the protoplasm set in which are essentially like those seen in the first division cycle of the monaster eggs except that the activity is not confined to one side of the egg but involves the whole or nearly the whole surface (fig. 6). Very frequently a rough division of the egg separates two blastomeres, (figs. F_5, F_6, or F_7) but this is accompanied by a great deal of protoplasmic streaming and the division is never permanent. Following the second division cycle, a third sets in. The movement of the second period is usually not completed by this time. The movements of the third period are even more severe, as a rule, than any of the earlier ones, and an endless number of bizarre shapes are seen. One very characteristic type seen is shown in figure 10. On such an egg little protoplasm droplets will be thrown off which do not fuse again with the mother egg, probably, because of the ectoplasmic layer has disintegrated by this time. Several more division cycles may follow (Wilson, '01 a, saw as many as 6 in one egg) but ultimtely disintegration results (fig. 9).

The behavior both of the aster and of the protoplasm, as has been described in the foregoing pages, may be taken as typical of what we would find, in any given set of monaster eggs. But extreme variations are often found from the courses described above. The figures together with the descriptions in the following section will give some idea of the extent of these variations and will show that they are variations in degree and not in kind.

In figures B_1 to B_6, the history of an egg is given in which the aster did not move to any appreciable degree. The egg was isolated when the controls were in the two-cell stage and it was a typical monaster (fig. B_1 drawn at 11.20). The egg underwent little change for the first ten minutes or so, then little chromosome vesicles began to appear and this was followed by a slight swelling of the ectoplasmic layer (fig. B_2, 11.42). This was accompanied by a slight change in the contour of the egg's surface. At 11.50, the rays of the aster had disappeared but the protoplasm of the egg was still somewhat irregular in outline (fig. B_3). At 12.08, an amphiaster was found in the egg. This lead to a division during which the binding membrane was greatly swollen in the division plane and seemed to undergo a sort of disintegration, as may be seen by figure B_5. The separation of the blastomeres was complete and was accompanied by the giving way of the cell wall, as was described for the eggs shown in figures 7 and 8. At 2.12, this egg had divided several times (fig. B_6), and the micromere which so often appears at this time is present (Painter '15).

This egg may be taken as typical in behavior in cases where the aster does not undergo a marked retreat. The swelling of the ectoplasmic layer and the movement of the protoplasm are both seen but neither are severe.

In figures C_1 to C_6 the history of an egg is shown, where the aster retreated only a short distance from the center. In figure C_1 the typical monaster egg is seen, as it was isolated at 11.17. By 11.25 the chromosome vesicles were visible, and at 11.50 the aster occupied a position somewhat excentric to the center of the egg. When the vesicles were forming a swelling of the ectoplasmic layer was observed (fig. C_2) and in figure C_3 we see the slight movement of the protoplasm. At 12.08 the monaster reappeared (C_4) and at 12.25 the movement of the protoplasm can be seen (fig. C_5). At 2.10 the egg had the condition shown in figure C_6.

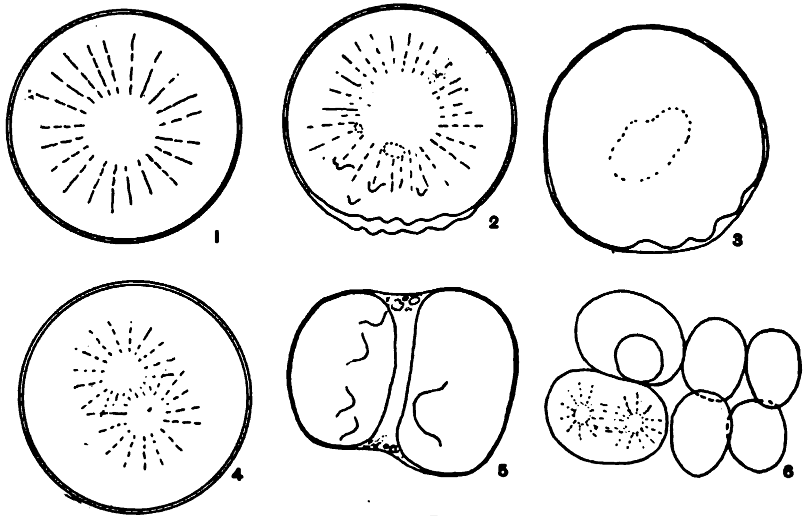
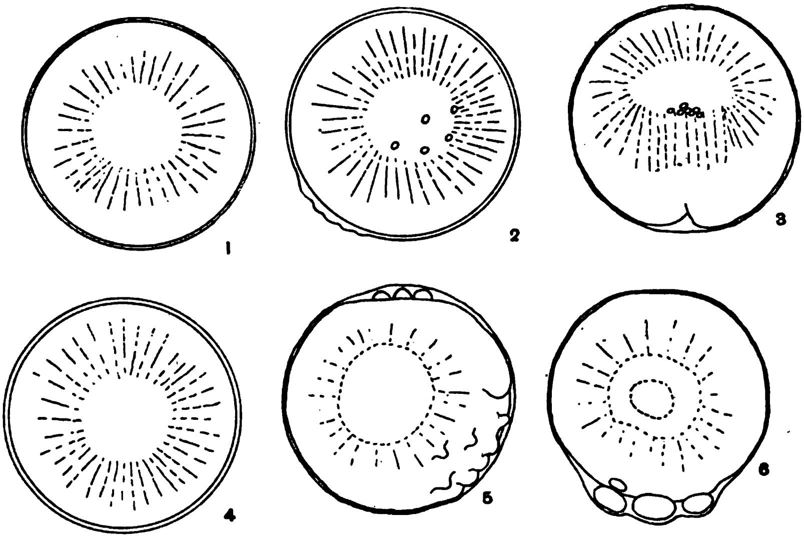


Fig. B

Figures D_1 to D_4 give the history of an egg in which the aster retreated farther than in the two cases just described. The swelling of the membrane together with the accompanying protoplasmic movement is clearly shown in figure D_2. An amphiaster appeared in this egg at the next division cycle (fig. D_4).

These three cases, taken from notes in which the history of dozens of similar eggs is recorded, well illustrates the great



C

Fig. C

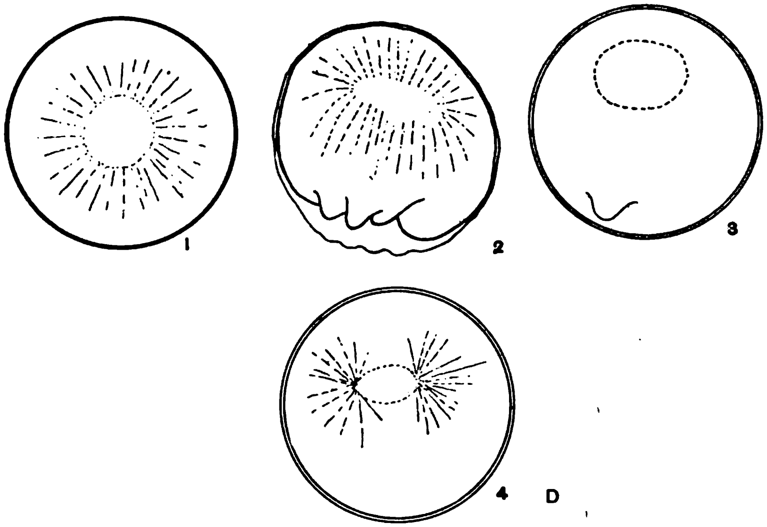


Fig. D

variability which the monaster eggs show in their behavior. These changes bear no direct time relation with the controls, the movements appear early or late, and continue for a shorter or longer time. (Direct evidence of this will be shown in the sections). In spite of this variation, however, there is a similarity in the behavior of all monaster eggs.

First there is always a swelling of the ectoplasmic layer before any sign of protoplasmic movement appears, and this swelling does not begin until the chromosome vesicles are being formed.

Second, the swelling invariably precedes the movement of the protoplasm.

Third, the severity of the protoplasmic movement seems to depend on the distance which the aster retreats from the center of the egg. When this retreat is extreme, we have a severe movement, figures 1 to 5, when it is not marked, there is little movement, figures B_1 to B_6 and D_1 to D_6.

An additional feature of the behavior of the monaster eggs should be emphasized. This relates to the ectoplasmic layer. It not infrequently happens that the swelling of the membrane is accompanied by the appearance of little granular irregularly shaped bodies in the otherwise homogeneous layer. This almost invariably happens in the two cell stage, as is shown in figures B_5 and in A_1 and A_2. When the two cells move apart, after the first division, this layer does not sink in as it does in the normal eggs. But it remains in the condition shown in the last figures named. Very frequently this swelling leads to the disintegration of the layer so that in the later stages, it is not present. As a result the blastomeres may become more or less scattered, as shown in figure B_6, but this does not seem to interfere with the formation of blastulae or gastrulae.

Internal changes

In taking up the study of sectioned material of monaster eggs, two main points were to be determined, first, the cause for the divergence in the later behavior of the eggs, and, second, the exact condition of the aster and chromosomes just prior to and during the periods of protoplasmic streaming. The detailed history of the aster and of the chromosome during the first monaster cycle had been worked out with such care by Mrs. Boveri that little was left to be added to this side of the problem.

Series of eggs, in which the per cent of monasters was high, were preserved at intervals of ten minutes or less, depending on the stage. The preservatives used were Boveri's picro-acetic mixture and sublimate acetic. After the usual treatment eggs were sectioned at 7 microns and stained in iron haematoxylin followed by light green. In practically all series whole mounts were made of a few of the eggs stained in borax carmine. These were very useful in orienting one as to the stages seen in sections.

One difficulty was encountered in the preserving fluids. While the chromosomes and cytoplasm, in general, gave beautiful preparations with both preservatives, the little protoplasmic processes did not retain their shape well. This difficulty was never fully overcome as these processes seemed extremely sensitive to both mechanical and chemical stimuli.

As was to be expected from the study of living eggs, a great deal of variation was found in the appearance and behavior of cell elements in the nine different series of eggs studied. The typical behavior of the eggs will be described first and other such variations as are illuminating will be taken up later.

Since a monaster phase is a step in the formation of an amphiaster, it is very difficult to distinguish true monaster eggs until the nuclear wall has disappeared and the aster has reached considerable size. In the earliest stages the centrosphere of the aster is small and deeply staining and the chromosomes are bunched on one side. This stage is quickly followed by an increase in the size of the centrosphere and a spreading of the chromosomes over one side of the aster (figs. 16, 17). Once reached, this condition persists a long time and corresponds, as M. Boveri has pointed out, to the equatorial plate phase in normal mitosis.

A careful study of these monaster eggs points to two types being present in all my material,[2] but whether the difference is due to capricious fixation or staining, or is of morphological significance, I am unable to say. Both types of eggs may be found side by side in the same section. In the one type (fig. 16) the center of the centrosphere is more deeply staining, and when one examines the structure under the highest powers, it will be found that this is due to the fact, that the meshes of the reticulum of which the centrosphere is composed, become smaller towards the center (fig. 18). This reticulum in my preparations is continuous with the aster fibers (fig. 18). In monaster eggs of the second type (fig. 17) the net-work of the centrosphere is uniform in size throughout the space so that the whole stains evenly. In other respects, the two types of eggs are alike. The chromosomes lie in a sort of half-shell around the centrosphere and they are always just about the same distance from the edge of the centrosphere (fig. I_1 to I_5 page 472).

As the controls finish the first division and enter into the second cycle, the centrosphere of the monaster eggs slowly increase in size. This carries the rays of the aster, with the chromosomes attached, nearer to the surface of the egg.

About the time half of the controls have divided into four cells, changes begin to appear in the monaster eggs. In part of them the chromosomes, which up to this time have been rod-like, undergo a splitting (fig. 20). This is found in eggs with the clear centrosphere. In the second type of egg, one frequently finds the centriole dividing as is shown in figure 19 and the spindle within the centrosphere may be clearly seen.

After the chromosomes have divided they rapidly approach the edge of the centrosphere, and following this, form little vesicles just as is found in the normal cleavage. At the same time the centrosphere becomes enormously increased in size (figs. 21 and 25) and the rays of the aster reach to the surface of the cell. As the chromosome vesicles fuse to form one nucleus, the rays of the aster appear to break up, (fig. 25) becoming granular, and gradually they entirely disappear. Traces of the lightly staining centrosphere may be found for some time, however. In only a few cases have I found evidence of the retreat of the aster in this type of egg. Two serial sections of such an egg are given in figures 13 and 15. The projections of the protoplasm formed during the movement are here clearly seen.

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[2] In one series of eggs, I have found, in addition to the two types of monaster eggs, here described, a third which is different and corresponds with the fan-asters ('Flächerkern') of the Hertwigs ('87) and others. Only part of the aster is present and there are only 18 chromosomes present. These eggs are beginning parthenogenetic development, but they soon disintegrate.

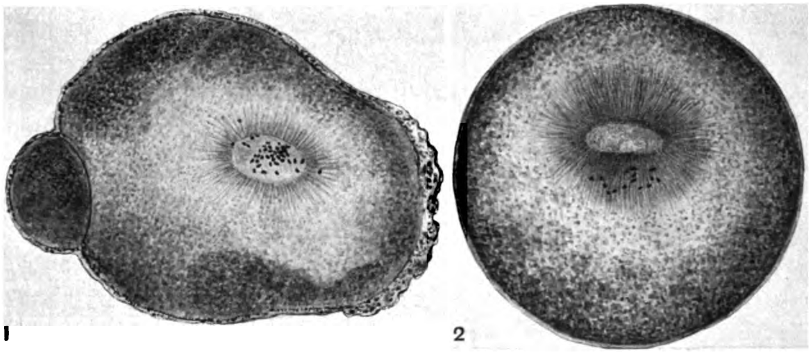

Fig. E

The eggs with a deeply staining centrosphere seem to undergo a different series of changes. Following the division of the centrioles (fig. 19) the centrosphere becomes elongated and moves toward one side of the egg (fig. 19 and text fig. E_2). After this two things may happen. The centrioles may separate completely and form a spindle between them. This process is usually accompanied by the formation of the peculiar spiral asters which I have described in another place ('16). The determining factor as to whether or not the spindle is formed seems to be the division of the chromosomes. If the chromosomes divide before the centrioles are far apart, then no separation appears to take place, instead nuclear vesicles are formed and these fuse to one nucleus as the astral rays disappear.

Quickly following on the first division cycle, the second one sets in, and the early appearance of the amphiaster is seen from figures 12 and 14. The spindle which results (fig. 24) appears normal except for the large number of chromosomes lying in the equatorial plate. I have not been able to count the exact number in the spindle, but corresponding stages in monaster eggs show 72 chromosomes (text fig. 9). In telophase stages the asters become extremely large, but I have found no sections through eggs just at the period when cleavage was taking place.

The triasters and tetrasters found in section and observed in the living eggs at this period, are to be derived, I believe, from these monaster eggs in which the centrioles divided early. They do not differ in section from similar eggs produced by dispermy.

Those monaster eggs in which the monaster persists in the second division cycle show considerable variation in appearance. In figure E_1 a rather common form is shown. The swollen ectoplasmic layer, and the irregular shape of the egg are reminants of the protoplasmic movement of the first division cycle. We see here the centrosphere surrounded by very short astral rays to which the chromosomes are attached. Figure 22 shows a later stage when the chromosome vesicles are beginning to fuse.

In figure F_1 to F_7 we have drawings of other types of monaster eggs. Figure F_1 is a later phase of the first monaster cycle. Figure F_2 shows a typical form of the second monaster. A clear defined centrosphere is not to be seen. Instead, the aster rays arise from a ill-defined area, and the chromosomes, instead of lying on one side of the aster, are here scattered all around the aster. Careful counts (fig. G) show about 72 chromosomes in the egg. Not infrequently, instead of one general center, there are four ill-defined centers from which the aster fibers arise. Figures F_4 to F_7 show sections through later stages. The irregular form of the eggs is due to the extraordinary movement of the protoplasm which follows the formation of the nuclear vesicles in the division cycle.

In later stages we may find the persistence of a single aster but more usually a great number are present as is shown in figure 23.

Discussion

With his characteristic insight, Boveri realized that the peculiar behavior of the monaster eggs was not to be considered as disintegration phenomena, but that each feature was to be found in normal cleavage. In the short paper already referred to, he calls attention to the main points of similarity in the external behavior of the eggs, and Mrs. Boveri extended the comparison to the cell elements. An exact correspondence was found throughout.

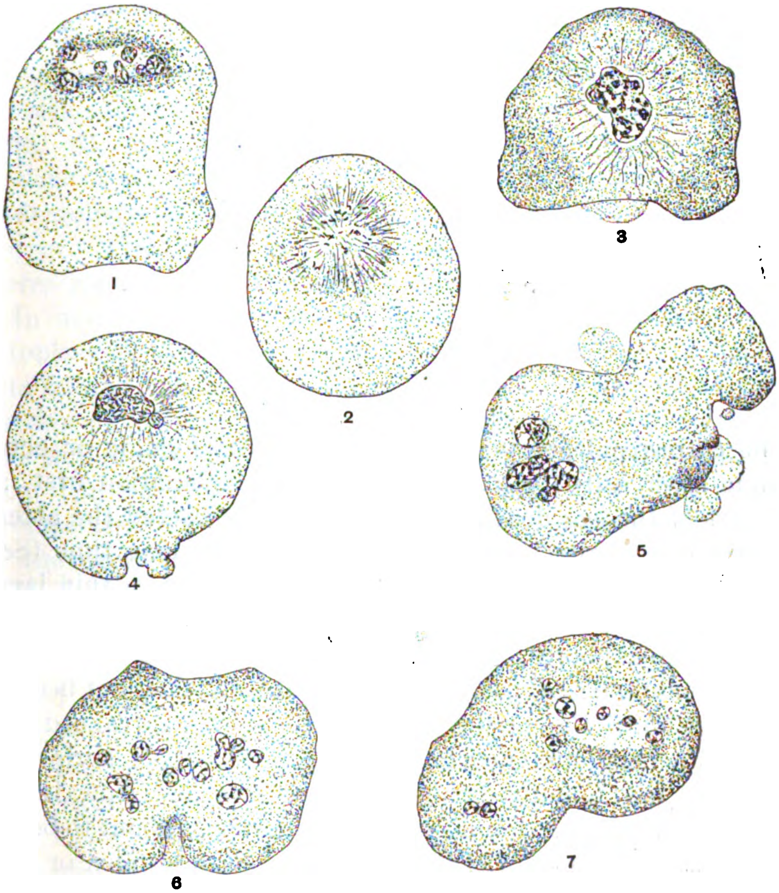

Fig. F

In the living egg, the similarity between normal and monaster eggs may be considered under three heads: the aster, the ectoplasmic or hyaline layer, and the granular cytoplasm.

The appearance of the asters, their growth, their changes in form, and finally their retreat towards the surface of the egg are phenomena so well known for the sea urchin egg that they call for no description here. The single aster passes through the same stages, as my description and figures will show.

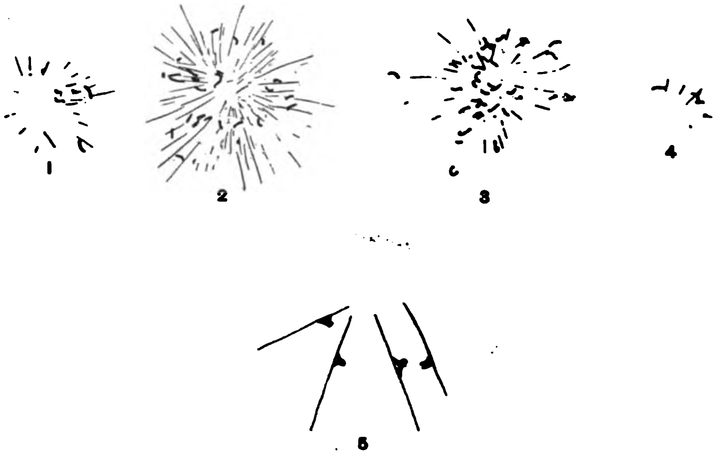

Fig. G

The correspondence in the behavior of the hyaline or ectoplasmic layer calls for a word by way of explanation first, as this part of the egg does not seem to have attracted the attention which it merits. It has been known for a long time (Selenka, '83 was the first to describe it) that there is a thin layer of ectoplasm surrounding the eggs of echinoderms, some molluscs, and ctenophores. This layer lies just beneath the fertilization membrane and, in the sea urchin at least, does not become prominent until the first division cycle is well advanced. It reaches its maximum development over the whole surface of the egg a few minutes before cleavage sets in. Now if one examines eggs with a dim light just as cleavage is about to begin, one will see that part of this layer has become swollen or blistered. This swollen area runs as a narrow girdle around the equator of the egg and just beneath it the cleavage furrow will appear. This is a very constant feature in Strongylocentrotus and has been noted by Boveri, Goldschmidt and Popoff ('08), and many others.

In monaster eggs we have the same swelling or blistering of the ectoplasmic layer, but it appears, not in a girdle between the two asters, but on the whole side of the egg opposite the chromosome side of the aster.

It is the third feature, perhaps, which forms the most striking parallel between the two types of eggs, namely, the behavior of the granular cytoplasm. In normal eggs, just beneath the swollen hyaline layer, the cleavage furrow appears. This gradually separates the egg into two cells, but the walls of the cleavage furrow are not as smooth as they are ordinarily figured. If any egg is observed very closely under moderate powers of the microscope, the furrow walls will be found to be undergoing changes during which little ridges and depressions appear. Finally, as the asters disappear the two new blastomeres round up and all active external movement ceases.

In monaster eggs, following immediately the swelling of the ectoplasmic layer the peculiar movement of the protoplasm sets in, and this leads to changes in surface contour and to the cutting off of protoplasmic masses, both of which indicate that this side of the egg is undergoing intense changes in surface tension. As the astral radiations disappear, these movements become less intense and eventually the normal rounded form of the egg is resumed.

It is clear that the force, or forces, which operate to bring about the separation of the two blastomeres with the attending movement of the furrow walls in normal cleavage are represented in the changes in surface contour and the cutting off of protoplasmic balls in monaster eggs.

In the second and later division cycles one finds in the monaster eggs, at least as far as the ectoplasmic layer and cytoplasm is concerned, the same correspondence to normal cleavage as described above. Thus, in those monaster eggs, which at the second division cycle shift over into an amphiaster, we find, in the cleavage plane, a swelling of the ectoplasmic layer and changes in surface contour (fig. A) which are similar to, but more severe than, those in normal division. And in those eggs where the monaster persists at the later cycle we have first, the swelling of the hyaline layer, then the movement and then the egg ceases to undergo active changes and resumes its rounded form more or less completely.

We thus see that the behavior of the monaster eggs, as aberrant as it appears at first sight, is similar to what we find in normal cleavage, both in the sequence of events and in the expression of the forces at work. And it gives us a far clearer picture of the intensity of the action of these forces, than that which we see in normal cleavage. No one has doubted that cell division was accompanied by changes in surface tension—any change from the spherical would necessitate this—but the formation of pseudopod-like processes and the general amoeboid movement of the protoplasm, lasting some time and involving a large part of the egg protoplasm, are expressions of a far more powerful force in cell division than normal cleavage would ever lead us to suspect.

One striking feature accompanying the protoplasmic movement, seen best in pigmented eggs, is the movement of the pigment granules (fig. 11). Both in Strongylocentrotus and Arbacia the pigment lies in the superficial layers of the protoplasm. During the protoplasmic streaming, in the first monaster cycle, most of this pigment tends to collect in the protoplasmic droplets (fig. 11). (This figure is drawn from an Arbacia egg). This fact demonstrates that accompanying the movement there is a flow of the cortical layers of protoplasm of the egg towards the side where the surface changes are most intense. This observation is of extreme interest because a number of authors have described a similar flow of substance toward the cleavage plane in other forms, and a good deal of importance has been given to this as a feature of cell division, i.e., Bütschli, and Conklin. A few illustrations will suffice to show how widespread the phenomena is.[3] Nussbaum ('93) observed that the brownish black pigment found in the entodermal and mesodermal cells of young Rana temporaria, tended to collect in a ring around the equator of the cell, and with division this pigment sank in and formed a cell plate between the daughter cells. Gardiner ('95) observed substantially the same thing for the cells of Polychaerus and Aphamistoma. Erlanger ('97) observed in the nematode egg, a movement of the protoplasm first towards the equator, thence inward and thence toward the asters. More recently Conklin ('02 and '05) has described in detail the vortical movements of the protoplasm in Crepidula, and Cynthia.

The intimate connection between the movement of the pigment and the changes in surface tension, during the division cycle of monaster eggs, points either to a common cause, or to the one being the effect of the other. And considering the widespread occurrence of the pigment movement in cells of other forms, it is clear that we are not dealing with a force peculiar to the sea urchin egg, but one common to many, perhaps all dividing cells. Any analysis which throws light on the nature of the surface tension changes, or their causes, is a decided advance.

The first step was to ascertain the cause of the swelling of the ectoplasmic layer. It seemed possible that this was due to some substance diffusing through the egg protoplasm, as Loeb ('95), and Robertson ('08) suggested. If this be true it might be through direct chemical action. I made a few preliminary experiments in Naples with this possibility in mind. Dilute solutions of HCl and NaOH were made in sea water. After the fertilization membrane had been shaken off, the acid was injected against one side of the eggs by means of Barber's apparatus. The ectoplasmic layer increased in thickness. The same results were obtained, however, with the sodium hydroxide, so I left Naples without reaching any conclusion on this point. Unknown to me then, Goldschmidt and Popoff ('08) had carried out a series of experiments, on sea urchin eggs, bearing on this general question, and their work sets the matter in such a clear light that I will review their paper in some detail.

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[3] For a full review of the literature up to 1902 see Conklin's work Karyokinesis and Cytokinesis. Since that time I have noted the following works. Fishel ('06) working with Arbacia pustulosa. McClendon ('09) working on Arbacia punctulata. Harvey ('10) working also with this same species.

In the ctenophores, the swelling of the ectoplasmic layer is very conspicuous, and Ziegler ('97) had assigned to it a constricting action in the cleavage of these eggs. This lead Goldschmidt and Popoff to investigate the origin of this layer, and the conditions under which it appears. They found that the layer appeared about the time the pronuclei of the egg were fusing and it steadily increased in thickness as the nucleus prepared for division. Just before cleavage set in, a girdle of swollen ectoplasm marked out the cleavage path.

When the water was allowed to evaporate from the dish in which the eggs were placed, the membrane grew thicker and this lead the authors to suspect that osmotic changes were responsible for its appearance. When eggs were placed in hypertonic sea water as the spindle was being formed, the layer greatly increased in thickness, at the same time numberless little processes of granular cytoplasm penetrated into the transparent layer, anastomosing and branching, giving the layer a vacuolated appearance. As a rule such eggs did not divide, but when a cleavage furrow did appear, the ectoplasmic layer did not sink in with it as it does under normal circumstances. In the space thus left, little radiating fibers (Zuglinien) appeared running from the granular cytoplasm to the layer. Eggs treated with hypertonic sea water showed only an extremely thin layer of ectoplasm in later stages. Also the authors reached the conclusions that the ectoplasmic layer, in later stages, is a true membrane and not an integral part of the egg.

These experiments show that osmotic changes cause the swelling of the ectoplasmic layer, in fact, that an increase in the density of the sea water causes the increase in thickness, while hypotonic sea water tends to inhibit its appearance.

Returning now to the monaster eggs, since the density of the sea water remains the same, we seem forced to conclude that there is an osmotic change in the protoplasm just beneath that portion of the ectoplasmic layer which swells later. In other words that preceding the active changes of form which the protoplasm undergoes, there are great changes in the osmotic relations of this side of the egg, as is shown by the swelling of the ectoplasmic layer. If we may safely compare the experiments of Goldschmidt and Popoff with our eggs, we should say that the movement was preceded by an increase in the density of one side of the egg.[4] This would carry with it, of course, a difference in the permeability of different parts of the egg.[5]

To what was this change in density due? Three possible sources could be suggested. First the aster, second the nucleus, and third the cytoplasm.

The aster. The movement of the aster was first considered as the cause of the movement, because the distance which the aster moved through the egg was a measure of the intensity of the movement. Thus, when the aster retreated well up against one side (figs. 2, 3, 4, 5) the movement was very severe, while, when the aster moved little or none at all (fig. B), there was a very slight movement of the protoplasm. It was possible to explain this fact in one of several ways. Later stages, however, did not support the view that the size or movement of the aster played any part, for the simple reason that, while the aster became often smaller and underwent little or no movement (fig. F_1 to F_3) the severity of the protoplasmic movement was greater than in the first cycle.

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[4] This is precisely what Butschli ('92) has maintained, and with him, a number of other investigators. Among the latest of these is McClendon ('10, '12). Robertson ('09, '13) has taken issue with McClendon and claims that, "In cell division the cleavage furrow is a region of low superficial tension, the poles of the egg regions of high superficial tension. Butschli and McClendon's view to the contrary is shown to involve a contradiction of the laws of molecular attraction of the liquids." (Robertson, Arch. f. Entw. Mech., vol. 35, p. 707). Robertson's views are based upon certain experiments with oil drops. "Drops of rancid olive oil were floated upon water, and threads wetted with solutions of strong bases, were laid across the drops. Division resulted owing to the equatorial diminution of surface tension resulting from the formation of soap" (Robertson, l.c., p. 692). In describing the behavior of the oil drops, he distinctly points out that there is a superficial flow of substance away from the equator towards the poles of the egg: "and violent streaming motions occur at the surface away from the thread and towards the opposite poles of the drop." (Arch. f. Entw. Mech., vol. 27, p. 30.) This is exactly the reverse of the condition found in the living egg, by a great number of investigators, including myself. Clearly, the conditions within the egg, are quite different from those in the oil drops with which Robertson experimented. This flow of substance towards the cleavage plane involves an increase of surface tension in this region, as Robertson points out, "for an increase in surface tension at the equator, such as Butschli imagines to occur would result in a streaming of material towards the equator" (p. 29, Arch. f. Entw. Mech., vol. 27). Robertson made the error of comparing the conditions within the oil drop (a homogenous body) with that of the living egg.

[5] See Lillie, '16.

The nucleus. The nucleus offered less difficulties as a possible source of the protoplasmic movement than the aster. The protoplasmic movement appears always just as the nuclear vesicles are being formed, and the increase in the number of chromosomes at each succeeding division cycle is accompanied by an increase in the intensity of the movement. Again, the fact that the movement is seen only on that side of the egg towards which the chromosomes are directed (in the first monaster cycle) points strongly to the latter as being in some way concerned with the movement. And in latter division cycles when the chromosomes cover the aster more or less completely, the formation of pseudopod-like processes involves nearly the whole egg's surface.

Assuming the nucleus to be the cause of the movement, it would be easy to explain the relation of the aster to it. Cytologists are in general agreement over the fact that the aster tends to exert a solidifying influence over the egg protoplasm, that is, cause the protoplasm within its influence to be less fluid. If this be true then parts of the cell farthest removed from the influence of the aster would be the first to be affected in case some substance was present which altered the surface tension of the protoplasm. In eggs where the aster retreated to one side, the whole opposite side of the egg would be free to move. In case the aster did not retreat, then we should expect to find little movement. Again in eggs, such as monaster eggs of the second or later division cycle, where the aster was small, we should expect to find here the greatest freedom of movement. This corresponds with the observed facts.

On the other hand, three independent workers (Wilson,'01 a, Ziegler '98 a and Mrs. Boveri) have reported that in enucleated blastomeres in which asters were present, the latter have the power of surrounding themselves with a certain amount of protoplasm, and in one case (reported by Ziegler) of actually dividing. While no mention is made here of the swelling of the ectoplasmic layer or of cytoplasmic movement, it seemed possible that both were present. In which case the chromosomes could not be regarded as causing the movement.

The cytoplasm. A consideration of the above facts lead me to take up the cytoplasm alone, as causing the movement. The difficulty here was that supposing the swelling of the membrane and the surface tension changes following were the result of certain products produced by the oxidation of the cytoplasm, then we should expect to find that the movement occurred in all the eggs at the same time. This is far from being the case; the egg protoplasm remains unchanged until certain processes are completed in the chromosomes and aster, and this may take place anywhere between the 2- and 8-cell stage of the control.

From the analysis of monaster eggs, it was clear that one could not determine which, of the three possible factors, was responsible for the protoplasmic movement, or whether perhaps all three were involved. Further progress could only be made when one or more of these factors could be eliminated.

An attempt to eliminate the aster from the problem was made by the use of the narcotic, phenyl urethane. The results of the experiments are given below.

EXPERIMENTS WITH NARCOTICS

During the course of an experimental study in which the eggs of Strongylocentrotus were treated with dilute solutions of the narcotic phenyl urethane, I noticed that eggs left a long time in this solution would finally undergo an irregular sort of cleavage (figs. H_1 to H_4). Furrows would appear in the egg's surface preceded by a swelling of the ectoplasmic layer, and two or more blastomeres would be cut off. Such divisions were not permanent as a rule. On examining these eggs with high powers I was unable to detect the presence of any radiations in the cytoplasm, although in the central clear area of the egg, a few very faint fibers were visible. A study of the treated eggs was undertaken in order to find out the changes which preceded this cytoplasmic division, with the hope of being able to throw light on the cause of the protoplasmic movement in the monaster eggs.

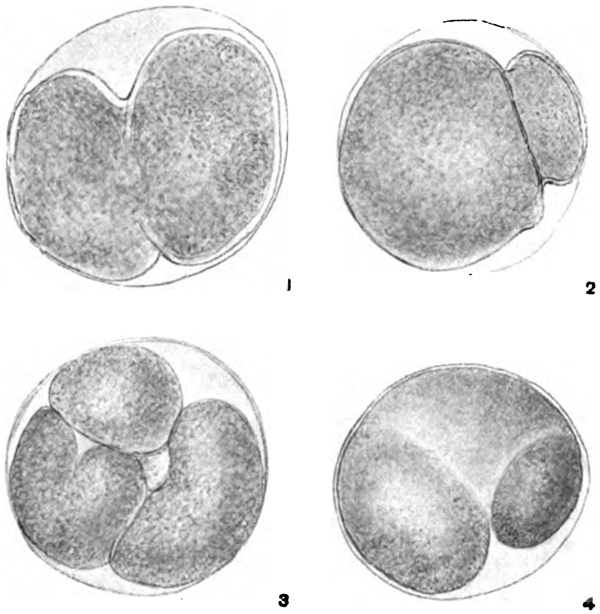

Fig. H

The present experiments were carried on at Woods Hole and the material used was the eggs of Arbacia punctulata. After fertilization, the eggs were allowed to undergo development for five to fifteen minutes and then they were placed in a 1/2000 N solution of phenyl urethane in sea water. The eggs were followed under the microscope, and portions both of the controls and of the experimented eggs were preserved at ten minute intervals either in strong Fleming or in sublimate acetic, since both of these fluids bring out the achromatic structures. Sections were made at 7μ, the stains used were iron haematoxylin followed by light green.

In the living egg, very little can be made out on account of the heavy pigmentation. In the following description observations from one set of eggs will be given. There was some variation in the different experiments, of course, but the behavior of the eggs was essentially the same in all cases. At the time the eggs were transferred to the narcotic, the male pronucleus was already visible as a very small globular body. After the eggs were placed in the solution, it increased very rapidly in size and slowly approached the female pronucleus. In part of the experiments, an ultimate fusion of the pronuclei took place, in others, the male and female elements remained separate. When the fusion did take place, it was very late, for the controls were in the stage when the aster becomes elongated into a crescent just before a spindle can be seen.

After the fusion of the pronuclei there was a rapid increase in the size of the nucleus due to swelling. In the event that a union did not take place, the male element increased greatly in size while the female pronucleus increased only slightly. Then the nuclear wall disappeared and in the egg one could see that the nuclear area showed traces of fibers. This condition persisted for a long time. At no period did one find cytoplasmic radiation. About the time the controls are in the 4-cell stage, the radiations in the nucleus disappear and following this the cytoplasm begins to undergo a labored division during which two or more blastomeres may be separated. Figures H_1 to H_4 show camera sketches of such eggs made from *in toto* mounts. Accompanying this division there was a decided tendency for the pigment to collect in the division plane, showing that there was a flow of the superficial protoplasm, just as there was in monaster eggs. In some few cases the division was permanent. In most eggs, however, after the cleavage furrows had persisted for some time, they would gradually fade out and the egg would resume its normal rounded form.

In studying the sections the main point to be determined was the condition of the eggs in which this division was taking place, especially was it desirable to see what had become of the aster.

Five minutes after the eggs have been transferred to the solution of the narcotic, one finds the condition shown in figure 26. The male pronucleus lies at some distance from the female element, it is small and, surrounded in every case in my preparations, by an area which is free from granules. Some sort of fluid seems to have collected around the male element. There is absolutely no sign of any cytoplasmic radiations at this time. Later stages show the approaching and touching of the male

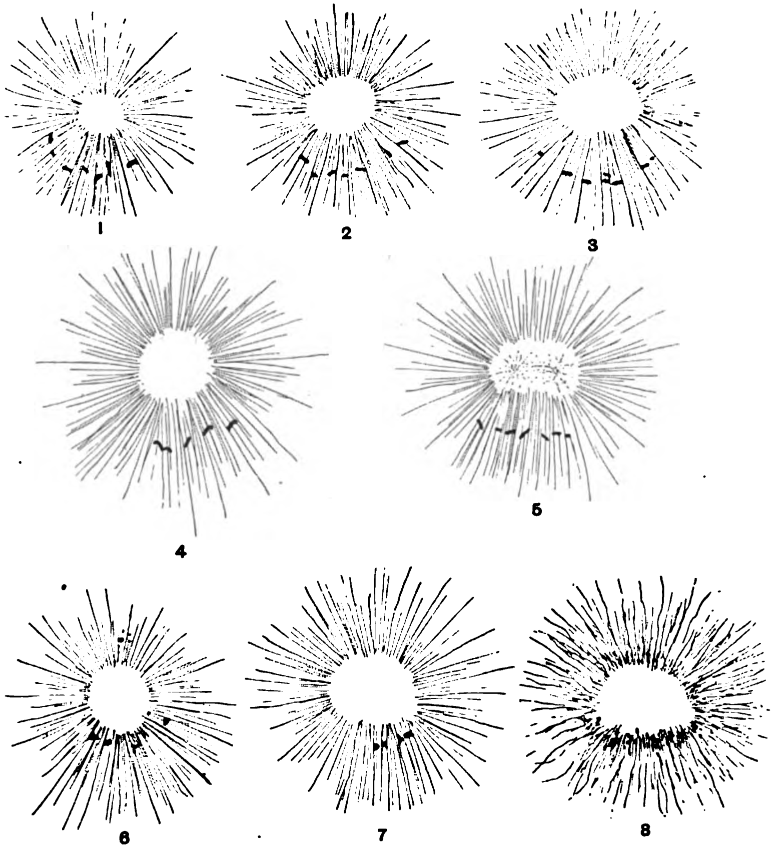

Fig. I

and female pronuclei. It is also interesting to note that the clear fluid area accompanies the male pronucleus up to the time of fusion, but after this it disappears. The swelling following the union of the pronuclei goes on rapidly and the nucleus becomes very large. At the same time, the chromatin collects into definite bodies and just before the nuclear wall disappears the chromosomes show very distinctly, lying on the linin net-work. As the nuclear wall is dissolving one occasionally sees a centrosome, (fig. 27), which appears to lie on the nuclear wall, and the fibers from this center appear continuous with the linin net-work in which the chromosomes lie. The division of the centrosome seems to already have taken place, for with the disappearance of the nuclear wall, a spindle is formed within the nuclear area (fig. 28). The whole nuclear cavity is filled with exceedingly fine parallel fibers and the chromosomes lie scattered in an irregular equatorial plate. Although I have studied these preparations with the best lenses I have been unable to detect a centrosome at either end of the nuclear area. At this time there are absolutely no cytoplasmic radiations to be found in the eggs of my preparations. Following this an irregular sort of division of the chromosomes seems to take place (fig. 30) and chromosome vesicles form at the two sides of the nuclear area.

In those cases where the pronuclei did not fuse, we find that the nuclear walls disappear at the same time. The chromosomes lie on a network which in some cases has a parallel structure, like that of a spindle (fig. 29). An irregular division of the chromosomes takes place here too and afterwards the chromosome vesicles are formed (fig. 31).

At the time when the nuclear vesicles are fusing into one or more nuclei, we find the movement of the protoplasm beginning. There is the greatest variation in the appearance of the eggs but they all agree in this that the cleavage furrow always cuts through the place occupied by the nucleus (figs. 32 and 33).

The point which I wish to emphasize is this, that at no time during the whole history of these eggs, is there any trace of cytoplasmic radiations. There is, in short no aster—though centrosomes may be present—and yet there is a flow of protoplasm into the cleavage plane and the eggs divide.

FURTHER DISCUSSION

As striking as these experiments with phenyl urethane may appear at first, the results were not wholly unexpected in the light of other researches. Wilson ('01 b) observed that when he allowed the ether to evaporate in the dishes where eggs were, that eventually these eggs would attempt an abortive sort of division.[6] During this time the asters were but feebly developed. And Teichmann ('03), repeating Wilson's experiments on Echinus microtuberculatus, remarks that he observed an egg divide after being treated with ether, in which he could observe no astral radiations. Neither of these authors gave the matter any closer study.

In Arbacia we have as typical a development of the aster as is to be found among animal cells, and yet the experiments with phenyl urethane show that in the total absence of these striking structures, the egg may undergo cleavage. Whatever the function of the aster may be, these experiments make it clear that a second factor (or set of factors) is at work which operates to bring about cytoplasmic cleavage. This division is accompanied by a swelling of the ectoplasmic layer and a flow of pigment towards the cleavage furrows.

To return to the question of the cause of protoplasmic movement, it is clearly demonstrated that neither the aster nor the processes involved in its formation (centripetal flow of ground substances, Rhumbler ('96), etc.) are responsible for the swelling of the hyaline layer or for the flow of pigment granules into the cleavage furrow or for the cleavage itself. This forms very conclusive evidence that these changes (at least the first two) in monaster eggs are due to another cause.

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[6] Warburg ('11) has shown that dilute solutions of phenyl urethane, such as I have used, do not materially alter the rate of oxidation of sea urchin eggs. Ether, however, (and a number of other narcotics) does diminish the oxidation rate. It is possible that the greater activity which the eggs of my experiment showed was due to this difference.

With the elimination of the aster from our problem, we still have in the nucleus, and in the cytoplasm, two possible sources for the behavior of protoplasm in monaster eggs. I have been unable to devise experiments which would give decisive evidence here. On the whole the mass of evidence is in favor of the nucleus, though we are utterly in the dark as to how it works. In addition to the evidence discussed on page 468 in favor of the nucleus, the experiments with phenyl urethane show that when cytoplasmic cleavage does occur, it cuts through the region where the chromosome spindle lay. The position of the chromosomes seems to play a part here.

As additional evidence we may point out that cytoplasmic cleavage rarely or never occurs in the absence of chromatin from the spindle. This is nowhere shown with greater clearness than in Boveri's paper "Zur Physiologie der Kern-und Zellteilung" ('97). In cells with tetrasters, cleavage only took place between poles in which chromosomes were present. Although shallow cleavage furrows might appear between asters where no chromatin was present, lasting division was never seen.

While at first sight the elaboration of Loeb's suggestion which Robertson ('08) has given seems plausible enough, there are difficulties to be met which are not easy to overcome. As is well known, Robertson suggested that some substance (cholin) diffused through the cell cytoplasm on the disappearance of the nuclear wall, and when this substance reached the surface of the egg, it formed soaps which lowered the surface tension of this part of the egg and hence brought about division.[7] If the swelling of the ectoplasmic layer and the phenomena following it were the result of simple diffusion, it seems probable that it would appear in all eggs about the same time. Far from this, as I have repeatedly pointed out, the period when the movement occurs is extremely variable and depends apparently on certain changes in the aster and chromosomes. If monaster eggs alone were to be considered, this objection might be met by assuming that the aster exercised a retarding influence on diffusion, but in those eggs treated with phenyl methane, in the absence of asters, the division of the egg was retarded until after the chromosomes had split.

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[7] Concerning Robertson's contention that cell division is brought about by a lowering of the surface tension at the equator of the egg, see footnote 4, page 467.

Further speculation of the subject is not desirable until we have more data upon which to go. The one outstanding fact is that there is a second factor entering into the cleavage of the sea urchin egg, a factor (or set of factors) characterized by a swelling of the ectoplasmic layer, great changes in the surface tension of the protoplasm, and a flow of the pigment towards the cleavage plane.

Is this second factor, which the monaster and narcotized eggs lead us to assume, something new? The answer is clearly in the negative. The monaster and narcotized eggs only allow us to see far more clearly the expression of this second force than normal eggs do, and furthermore, permit us to harmonize the widely differing views of other investigators.

As long ago as 1876 Butschli gave a mechanical theory of cell division based on movements of the protoplasm, and since that time a number of authors have observed movements towards the cleavage plane, which might be of significance in cell division. Most conspicuous of these are the observations of Erlanger ('97) who studied living nematod eggs at Butschli's suggestion, (see Butschli '00) and the works of Conklin, who finds in "the vortical flow of cell substance" (Karyokinesis and Cytokinesis, p. 100) one of the most important factors in cell division.

From a different standpoint we have Loeb's ('95) suggestion:

> Ich stelle mir nun vor, dass an der Oberfläche des Eies, möglicherweise in demjenigen Meridian oder Kreise, dessen Ebene die beiden Strahlensysteme der Centrosome von einander trennt, sobald die Kerntheilung physikalisch abgelaufen ist, Ausbreitungserscheinungen stattfinden. Dieselben führen zur Bildung von in Bezug auf diese Ebene symmetrischen Wirbelbewegungen.

Rhumbler ('96) finds in the growth of the membrane (gesteigertes Membranwachstum) a factor which assists the asters in cell division. This phase of cell division is more fully elaborated in a later paper (Rhumbler '99).

Zeigler ('98 b) interpreted the swelling of the ectoplasmic layer in ctenophores in a different way, as I pointed out on page 466.

More recently we have works which approach the problem from the standpoint of physical chemistry. Foremost among these is Lillie's work, who finds that there is a distinct change in the permeability of the cell during each division cycle, and as he points out, this would be accompanied by changes in surface tension (Lillie '16).

A moment's consideration will show that these three widely differing views on cell division may be united by this study of monaster eggs. The swelling of the ectoplasmic layer is just the same phenomena described by Rhumbler and Zeigler. No where is the "vortical flow of cell substance" more clearly seen than in monaster eggs. And finally the great changes in surface tension which monaster eggs undergo indicate a difference in density (and of necessity permeability) on the several sides of the egg. In monaster eggs, however, these phenomena follow each other in regular order, and appear to be due either to a single factor, or several factors working together. Perhaps the whole series of events may be traced to a difference in the density of the protoplasm on the side of the egg opposite the chromosomes in monaster eggs, or to a dense girdle of protoplasm in the case of normal eggs. Later researches must throw light here. But a distinct advance has been made, I believe, when we are able to harmonize the discordant views of so many investigators, and show, as I think the above analysis does, that they were all dealing with different expressions of the same force.

THE CENTROSOME

The present work throws no new light upon the origin and nature of the centrosome, but certain phases in the behavior of the division mechanism call for special mention here.

Normally, the monaster condition lasts for a very short time in the division cycle, only so long as the centrioles are undivided, according to Boveri (Zellenstudien IV). In monaster eggs this period may be prolonged through a whole division cycle without seriously disturbing the division mechanism. We are totally unable to suggest the cause of this delay in this division (or the separation of the centrioles), but it seems clear that the abnormal condition may be overcome at any period in the division cycle. Thus a few eggs recover before the controls have divided. In others the centrioles divide and separate when the controls are preparing for the second division. In others the whole division cycle may be passed through, and this is what occurs in the majority of the eggs. Or finally the separation may be suppressed until the third or fourth division cycle has been passed through; or, even permanently.

The suppression of one division does not affect the centrioles injuriously, in the majority of the eggs, but frequently in eggs which have passed through several cycles in the monocentric condition polyasters suddenly appear. How are we to explain such cases? Two possibilities present themselves. If we assume that centrioles be at the focal point of the rays, either the centrioles have divided at each division cycle but have failed to separate, a view for which I have no evidence to support, or, as the inhibiting factor is removed, the centriole have been excited to rapid division. The second effect may be experimentally obtained by treating eggs with various poisons.

Another feature which should be mentioned is the increased size of the asters in those eggs which have passed through one division cycle and have then formed amphiasters. The appended figures K_1 and K_2 show the size of the normal asters K_2 (in the first division) and the spindle of an egg with a monaster history K_1. The difference in size at the same phase of development will be apparent on the inspection of the figures. Boveri along with a number of other investigators has pointed out that there is a constant relation between the size of the aster and the protoplasm it controls. That is, a monaster would be larger than a single aster of an amphiaster were the egg the same size in both cases. These spindles with a monaster history form an exception to this rule. Again we are confronted with two possible explanations. If we adhere to the old view of a definite archoplasmic substance, then we can say that while the centrioles may have failed to divide, this substance has increased in amount. Hence at the second division cycle, the asters are larger. The more likely explanation, however, seems to me to be in the relative amounts of chromatin, in these eggs. A number of investigators have maintained that the increase in the size of the asters is due, in part at least, to the absorption of nuclear sap, which is set free in the egg cytoplasm when the nuclear wall disappears. In these eggs with larger asters, we have just double the number of chromosomes that are found in the first spindle. If the nucleus contributes to the growth of the aster, the large size of the asters in eggs with a monaster history is not difficult to explain.

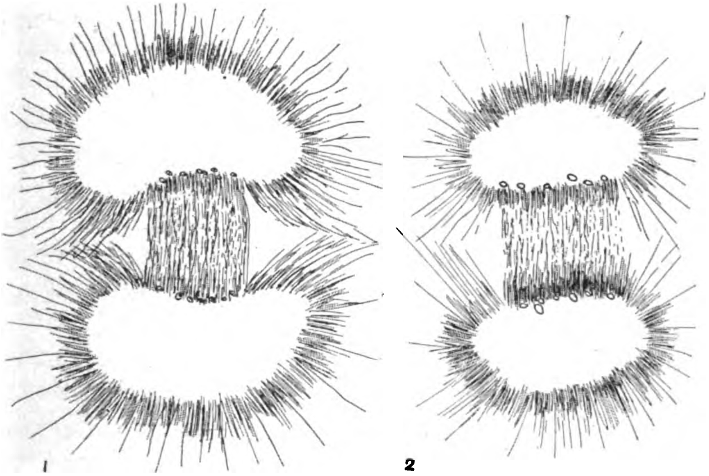

Fig. K

THE ASTER AND CYTOPLASMIC DIVISION

The centrosome with its surrounding aster has been assigned such a prominent rôle in cell division by most investigators working on animal cells that it is very desirable to arrive at some conclusion as to its function on the basis of the present work. At first sight the present investigation seems to minimize the importance of the aster in cell division. Indeed, I believe that it has been clearly shown, especially by the narcotized eggs, that in the total absence of the asters, the eggs may divide. To conclude from this that the asters do not have a rôle, and a very important one, would be a very serious mistake. For the narcotized eggs and, particularly the monaster eggs, demonstrate that while a force, independent of the aster, is at work within the cell, this force produces little more than striking changes in surface tension which do not accomplish any definite regular result. A directive structure is lacking in monaster and narcotized eggs, and this fact I believe, points out to us the principal function of the aster in the sea urchin egg.[8] A number of facts point to the correctness of this view.

First of all, the work on enucleated blastomeres in which asters are present show clearly that each aster has the power of surrounding itself with a certain amount of cytoplasm. When these asters are near the surface, furrows may appear around or between them, a phenomenon which can only be understood when we assume that the area around each aster is more solid than the outlying cytoplasm. This gives us the necessary mechanism for the directing of this second force which causes surface tension changes.

Again the variation in the intensity of the protoplasmic movement in the first monaster cycle can best be explained by assuming that the aster acts as a center of more solid protoplasm. Thus when the aster undergoes no retreat (fig. B_2) the force which produces the movement does little more than cause the ectoplasmic layer to swell, since the protoplasm is held fast until after the critical period has passed. Figure D_2 shows an egg where the aster retreated part way to the surface. Here the protoplasm released from its control is free to undergo a certain amount of movement and finally, in those commonest cases in which the aster moved until it lay well up against one side (fig. 1, etc.) the movement was most severe.

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[8] This applied to cytoplasmic division.

Undoubtedly the severe movement of the protoplasm during the change of monaster eggs (figs. A_1 and A_2) is due to the same cause. As I have pointed out, the spindle usually lies somewhat excentrically in these eggs with the result that the side farthest removed from the spindle shows the greatest amount of surface tension change. And in those eggs in which the monaster appears at the second division cycle, the severe movement, while due, perhaps to some extent to the increased number of chromosomes, is also to be correlated with the feeble development of the astral system.

These facts taken together with the evidence from the narcotized eggs point unmistakably to the asters as regulative centers during cell division centers, which determine the channels in which the second factor will operate.

BEHAVIOR OF THE CHROMOSOMES

A number of authors (Boveri, M. Boveri, Wilson et al.) have pointed out that monaster eggs demonstrate that the chromosomes do not require two opposing centers to pull them apart in order for them to divide and separate; the process is autonomous. A close study of the behavior of these bodies reveals several points of interest.

When the chromosomes become scattered over one side of the aster, they all come to be at about the same distance from the edge of the centrosphere (fig. H_1) and are attached to it by means of fibers. The relation is established very early, and, corresponds to the equatorial plate phase of normal division. Following this, the aster undergoes a slow increase in size (figs. H_2 to H_4) which causes the edge of the centrosphere to approach nearer to the periphery of the egg. The chromosomes are carried along on their fibers, so that they come to be farther and farther from the middle of the centrosphere. A close inspection of figures H_1 and H_5 will show that in all these cases the distance between the edge of the centrospheres and the chromosomes remains about the same.

This relation between the chromosomes and the edge of the centrospheres is retained until the chromosomes begin to split. As soon as division begins, the chromosomes rapidly approach the edge of the centrosphere (figs. H_6 to H_8).

A close study of the dividing chromosomes shows that the splitting takes place in one of two ways. Sometimes we find a clear line appearing between the two halves (fig. G_2) which separate more as they approach the centrosphere. More usually, however, the splitting is accompanied by a twisting of the two halves (fig. G_2) about each other and the formation of V-shaped figures. In either case, both halves retain their attachment to the same fiber, and this condition persists until the chromosomes have reached the edge of the centrosphere, in the vast majority of cases. Here and there, however, the two halves appear to be attached to different fibers, as they approach the sphere.

While no light can be thrown on the cause of the chromosome division, it should be pointed out that it appears entirely independent of the condition of the aster. The latter may be large or small, round or sunken in, or the centrioles may even be undergoing division. The chromosomes and the asters seem totally independent in their behavior.

By carefully measuring the distances between the chromosomes and the edge of the centrosphere, I have found that this is approximately the same in all monaster eggs in which the chromosomes are undivided. There is no sign of any movement of the chromosome until a split appears. Then the approach to the edge of the centrosphere is very rapid and few intermediate stages can be found. The division of the chromosomes seems to give the necessary stimulus which causes them to approach to the centrosphere.

This fact that no movement of the chromosomes takes place until after they have divided has an interesting bearing on the question of the forces at work here. As is well known, a number of theories have been advanced to account for the chromosome movement. Of these, three may be considered the more important. (1) Contractibility of fibrillae. Klein ('79), Van Beneden ('83), Boveri ('88), etc. (2) A pushing apart of the chromosomes; Growth of central spindle, Drüner ('95) etc., or Watase's ('93) ingenious suggestion. (3) Possibly carried along by protoplasmic movements, Bütschli ('76), or by chemotaxis Strasburger ('93). Most of the later writers on the subject have been inclined to Bütschli's view. Wilson ('95), Griffith ('99), Conklin ('02 and '05).[9]

Of the many objections to the contractile hypothesis, that urged by Wilson, and by Griffith seems the most cogent. In sea urchin eggs after the chromosomes have come to lie against the edge of the centrosphere, they spread out over its surface, a fact irreconcilable with the contraction theory. In monaster eggs this spreading of the chromosomes is seen exceptionally well.

Any hypothesis which attributes the movement of the chromosomes to interzonal fibers, can not explain the monaster eggs, since no such structures are present. Yet the chromosomes approach the centrosphere with rapidity and certainty.

This study of monaster eggs has brought to light no new facts inconsistent with the view that the chromosomes are carried to the aster by the flow of protoplasm. For as the chromosomes divide, the aster begins a rapid increase in size. It is interesting to note that no matter how long the chromosomes remain undivided, no great increase of the aster occurs until they are cleaving. There is a close connection here between the two phenomena.

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[9] The question of chromosome movement, which was so much discussed in the nineties, has recently received little attention. For a full review of the literature of this earlier period see Wilson's 'The Cell,' pp. 70–77, also Conklin's Karyokinesis and Cytokinesis. Although written some twenty years ago, Wilson's remarks still seem pertinent: "In any case, I believe that no satisfactory hypothesis can be framed that does not reckon with the chemical and physical changes going on in the centrosphere, and take into account also the probability of a dynamic action radiating from it into the surrounding structures." ('The Cell' page 77.) Such a theory has yet to be advanced, but several attempts have been made in this direction. See Lillie's various studies on Cell Division. Also Hartog ('14).

‘SPINNING’ OR ‘FILOSE ACTIVITIES’ IN THE LIGHT OF MONASTER EGGS

The protoplasmic movement of monaster eggs is similar, in several respects, to the ‘spin-thread’, or ‘filose activities’ observed by several investigators (G. F. Andrews ’97, E. A. Andrews ’98, Coe ’99, C. B. Wilson, ’99) in the polar body formation and in the cleavage of nemertean eggs. Also in starfish and sea urchin eggs as described by Mrs. Andrews.

As far as the protoplasmic activity of Cerebratulus is concerned, I believe the movements so well described by C. B. Wilson, and which I have myself observed on this same material, may be directly compared to those found in monaster eggs, since: first, they are found in the region of the cleavage furrow; and, second, are found just when cytoplasmic division is taking place, or has just been completed. So far as I am aware, nothing comparable to the swelling of the ectoplasmic layer has been described for the nemertean egg.

The ‘filose activities,’ described by Mrs. Andrews, were not limited to the division plane, but occurred at any place on the eggs surface and at any time in the division cycle. What relation these general activities bear to the periodic movements, described in this paper, is not altogether clear. The work of Goldschmidt and Popoff suggests a possible explanation. They observed that when eggs were placed in hypertonic sea water, the protoplasm lying just beneath hyaline layers underwent changes in form, during which little threads were formed; these branched and anastomosed, just as Mrs. Andrews described. Under the conditions which Mrs. Andrews’ work was made, it seems very likely that an evaporation of sea water took place, with the consequent increase in the density of the sea water. This may have been the primary cause for the activities which Mrs. Andrews observed. In this event, the periodic movements during certain phases of the division cycle, and the continuous activities, would be similar in so far as they are responses of the cytoplasm to the same stimulus, namely, changes in density. In the one case, however, the changes come from within (monaster eggs), in the other, from without.

SUMMARY

1. A study of monaster eggs, living and in sections, has been made.

2. It has been shown that, at each division cycle, the egg protoplasm is affected in three ways: (a) first there is a pronounced swelling of the ectoplasmic layer, (b) this is followed by intense changes in surface tension leading to the formation of pseudopod-like processes, (c) and accompanied by a flow of the superficial protoplasm toward the area where the streaming is taking place.

3. The intensity of the protoplasmic movement varies with the position which the aster takes at the end of the division cycle.

4. A comparison shows that the behavior of monaster and normal sea urchin eggs is similar.

5. The changes in the egg protoplasm are preceded by definite changes in the aster and in the chromosomes, and an analysis of the behavior of the eggs is made to determine whether these phenomena are due to the aster, nucleus, protoplasm, or to a combination of these.

6. No decisive evidence can be given to prove which part of the cell is concerned, but many facts point to the nucleus.

7. The swelling of the ectoplasmic layer may be produced by treating eggs with hypertonic sea water.

8. An attempt is made to eliminate the aster as a possible source for the behavior of the monaster egg, by treating eggs with phenyl urethane.

9. The experiments show that, in the absence of asters, the eggs may divide, the division being accompanied, in some cases, by a swelling of the ectoplasmic layer in the cleavage plane and a movement of the protoplasm into the cleavage furrow.

10. These facts eliminate the aster (and the processes which presumably lead to their formation, centripetal flow of protoplasm, etc.) from the problem.

11. All evidence points to the nucleus as the source of a second factor in cell division, a factor (or factors) which finds its expression in, a swelling of the ectoplasm, great changes in

surface tension and a flow of the cortical layers of protoplasm toward the cleavage area.

12. It is pointed out that this nuclear factor is not a new conception, but that its various expressions have been emphasized in turn, by a number of different investigators, working on different forms.

13. Evidence has been given to show that, as far as the cytoplasm is concerned, the asters play the rôle of regulative centers during cell division, that is, by forming denser areas they restrict the action of the nuclear factor to a prescribed area.

14. The history of the chromosomes is followed.

15. It is suggested that the 'spinning activities' or 'filose activities' of Cerebratulus eggs is similar to the protoplasmic movement of monaster eggs. In the case of the 'filose activities' described by Mrs. Andrews it seems possible that they are responses of the irritable protoplasm to a stimulus similar to that in the monaster eggs, but coming from a different source.

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EXPLANATION OF PLATES

Owing to the fact that this study has been made at different places, the drawings are not all of the same magnification. All the drawings up to and including figure 26, plate 4, were made, or completed, by Miss L. Krause of the Osborn Zoological Laboratory, Yale University, New Haven, Conn.

PLATE 1

EXPLANATION OF FIGURES

All drawings, with exception of 1 and 2, are from free hand sketches of Strongylocentrotus eggs.

1. Camera sketch of egg showing swelling of the hyaline layer.

2 to 5 Various stages of the protoplasmic movement in monaster eggs. Figure 2 is from a camera drawing.

6 Protoplasmic movement of second division cycle.

7 and 8 Dividing monaster eggs.

9 Disintegrating monaster egg.

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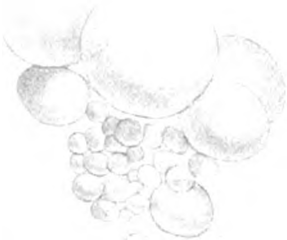

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PLATE 2

EXPLANATION OF FIGURES

All figures are from Strongylocentrotus except 11. All drawings were made with a camera lucida except 10 which is from a free hand sketch. Magnification varies as noted below.

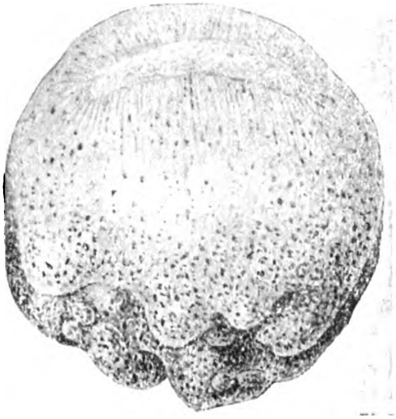
10 Protoplasmic movement in later division cycle. × 1100.

11 Protoplasmic movement in Arbacia egg showing migration of pigment. × 1200.

12 and 14 Monaster eggs beginning second division cycle. × 1200.

13 and 15 Consecutive sections through a monaster egg during the protoplasmic movement. × 1000.

10



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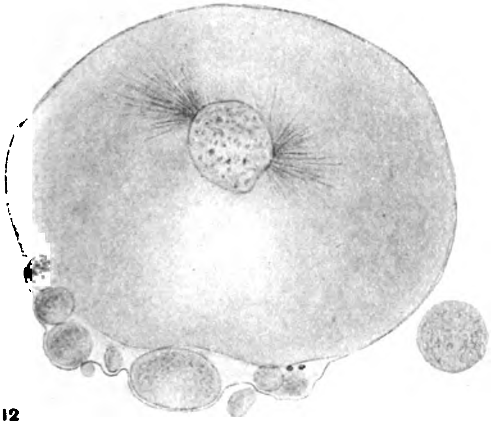

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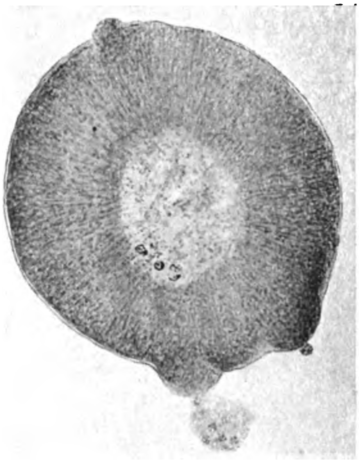

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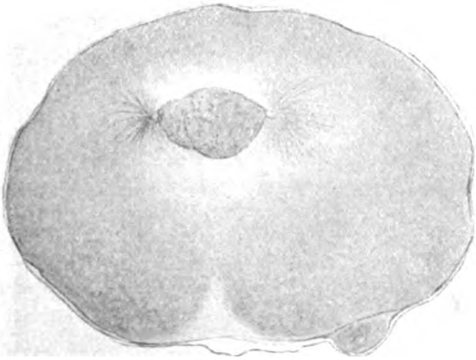

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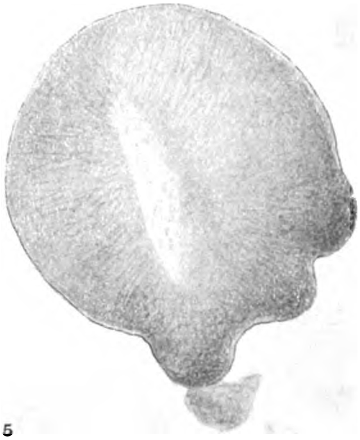

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PLATE 3

EXPLANATION OF FIGURES

Camera drawings of sections. Magnification same as noted below. All eggs are from Strongylocentrotus.

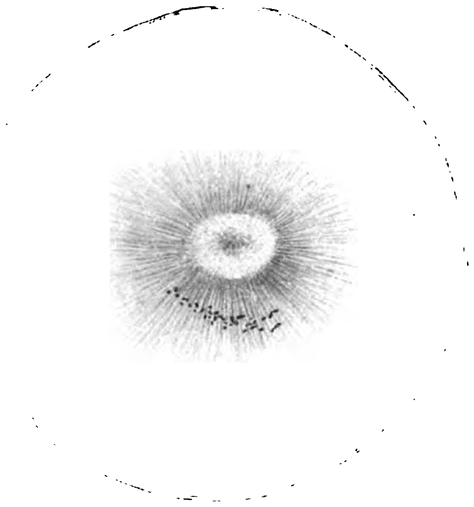
16 and 17 showing the two types of monaster eggs found during the first division cycle. × 1900.

18 Detail drawing of aster. × approximately 3000.

19 Monaster egg showing division of the centrioles. × 1500.

20 Monaster egg showing division of the chromosome. × 1900.

21 Monaster egg showing fusion of chromosome vesicle. × 1500.



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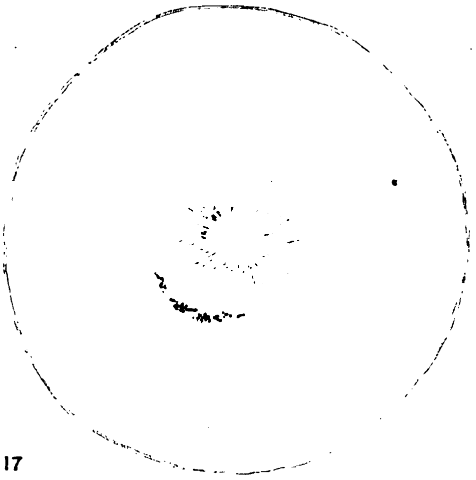

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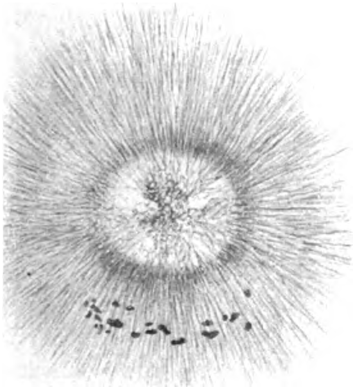

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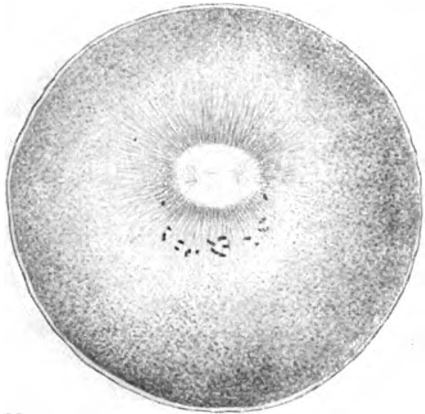

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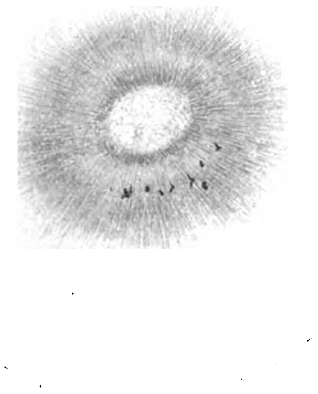

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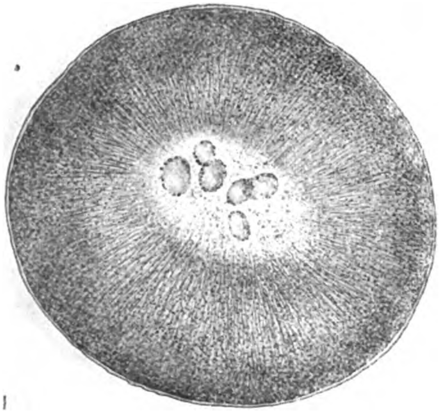

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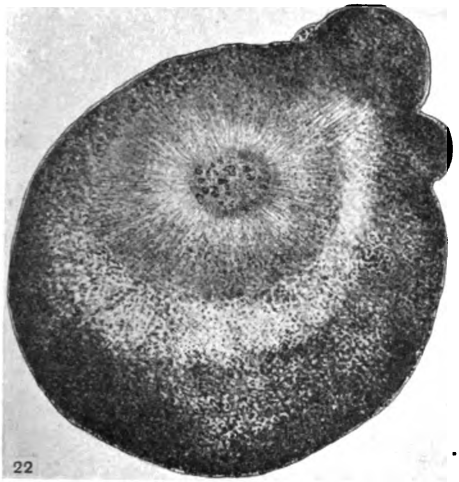
PLATE 4

EXPLANATION OF FIGURES

Figures 26 and 27 from Arbacia. The rest from Strongylocentrotus. All drawings made by means of a camera lucida.

22 Monaster egg in later division cycle. × 2100.
23 Monaster egg showing multiple asters. × 1600.
24 Spindle in second division cycle of monaster egg. × 1900.
25 Monaster egg showing chromosome vesicles. ×2100.
26 Arbacia egg treated with a narcotic showing pronuclei. × 2100.
27 Arbacia egg treated with a narcotic showing centrosome. ×2100.



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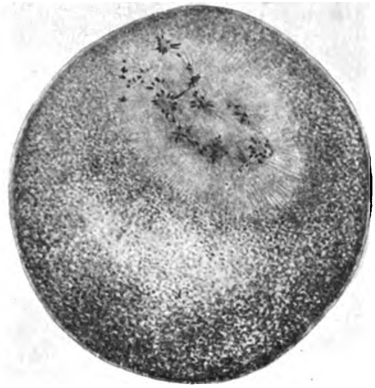

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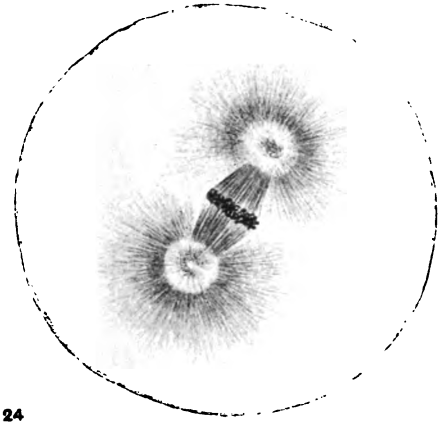

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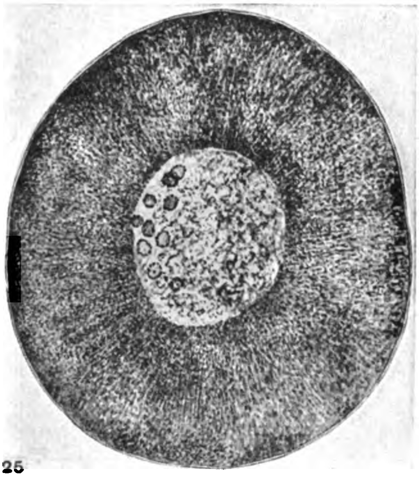

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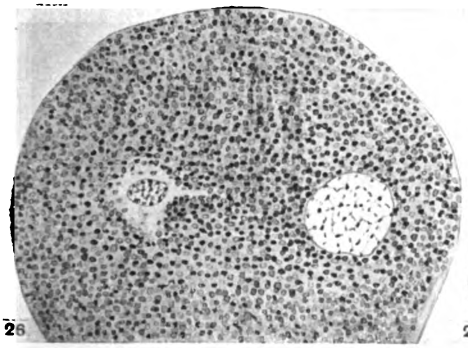

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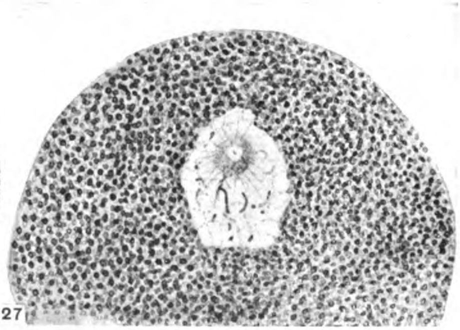

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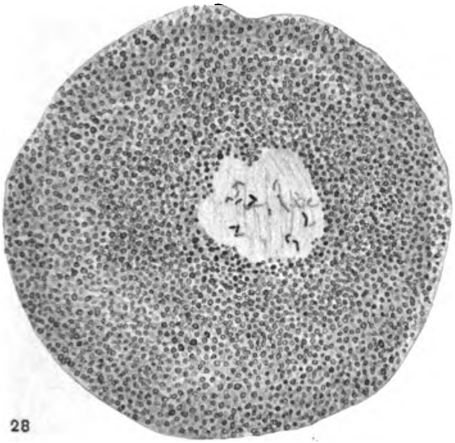
PLATE 5

EXPLANATION OF FIGURES

Drawings from sections of Arbacia eggs treated with narcotic, made with the aid of a camera lucida. Magnification approximately × 2100.

28 Egg showing nuclear spindle with absence of asters.
29 Egg in which the pronuclei did not fuse before the nucleus was dissolved.
30 Egg showing the division of the chromosome.
31 Egg showing the formation of chromosome vesicles.
32 and 33 Eggs showing cytoplasmic cleavage in narcotized eggs.



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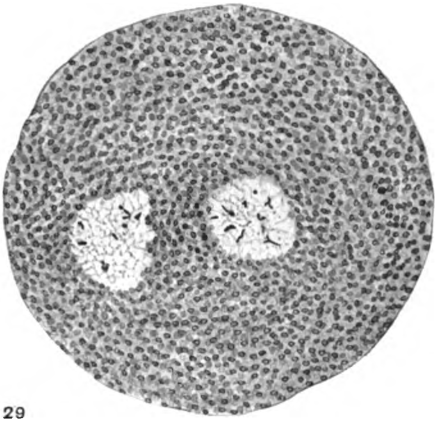

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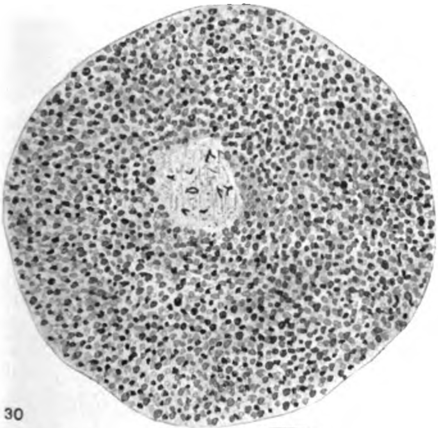

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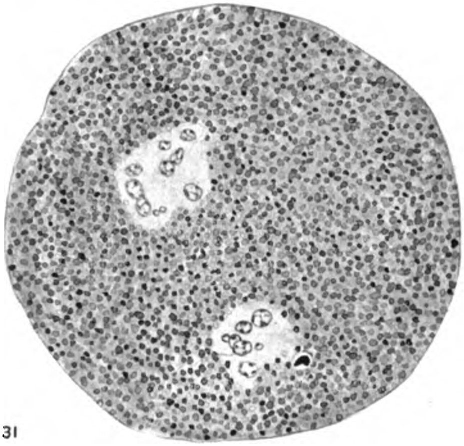

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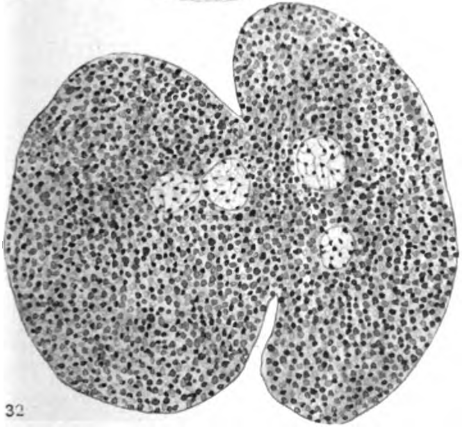

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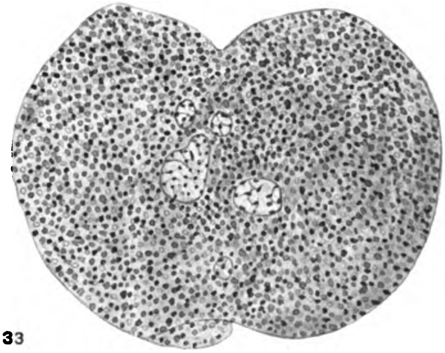

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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EXPERIMENTS ON THE DEVELOPMENT OF THE SHOULDER GIRDLE AND THE ANTERIOR LIMB OF AMBLYSTOMA PUNCTATUM

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THIRTY-THREE FIGURES

1. INTRODUCTION

Experiments on the transplantation of limbs have been carried on in connection with various problems: a) on the development of nerves (Banchi, '05; Braus, '05, and Harrison, '07); b) on the question of orientation and laterality (Harrison, '17); c) on the question of the development of the shoulder girdle (Braus, '09), the rudiment of which is intimately bound up with that of the limb. It is with these last experiments that we are particularly concerned, since they have a more specific bearing on the results of the investigations set forth in this paper.

Although the intimacy of these two systems—shoulder girdle and limb—during development and differentiation led Wiedersheim ('92) to conclude that girdle formation is possible only under the formative influence of the free extremity, the experiments of Braus showed, in part, the lack of interdependence of these systems. Braus found that the removal of the fore-limb buds of Bombinator included the tissue from which the central or glenoid portion of the girdle develops, and that only the distal parts—suprascapula and epicoracoid—were formed following such an operation. The differentiation of these isolated girdle elements from unremoved blastema in the absence of the developing appendage demonstrated their independence of the free extremity. Confirmation of this observation was made on Amblystoma by Harrison ('18), who found, however, that as development proceeded these two separate distal elements gradually approximated each other until, in a larva which was kept alive eighty-five days after operation, they had become united into a single cartilage.

The formation of the suprascapula and coracoid in the absence of the glenoid portion of the girdle demonstrates that their rudiments are already determined at the time of the operation, and that, while they eventually grow together, their unremoved rudiments are nevertheless not capable of restoring the missing parts, viz., the scapula, all or only a portion of the procoracoid, and the shoulder joint, the rudiments of which are removed in a typical limb-bud extirpation.

Braus ('09) further found that when a limb bud is transplanted to a heterotopic position, a *complete* shoulder girdle of one-third to two-thirds the size of the normal develops at the place of implantation. From this he concluded (page 271) that the shoulder-girdle rudiment constitutes an equipotential restitution system.

According to this conclusion, totipotency is restricted to those girdle-forming cells which become implanted along with the limb bud, for, as has already been pointed out, the unremoved blastema can develop only into those parts the rudiments of which are already determined at the time of operation. The formation of a reduced girdle, with all its components, from cells which, in the normal environment, give rise to only the more central parts, would show that in their normal surroundings their prospective potency is greater than their prospective significance.

The results of the experiments set forth in this paper seem to necessitate for Amblystoma, however, an interpretation different from that which Braus placed on the results of his experiments.

This investigation was taken up at the suggestion of Prof. R. G. Harrison. It gives me pleasure to express here my thanks to Dr. Harrison for the guidance he has given me during its completion.

2. NORMAL ANATOMY

In order that the experiments may be more fully understood, a description of the normal girdle will first be given. Chondrification of the girdle is practically complete in a larva about twenty days after the closure of the medullary folds. The girdle then consists of a cartilaginous structure lying within the body wall and extending from the lateral aspect of the third myotome almost to the mid-ventral line (fig. 6). It is made up of the following components: a) the suprascapula, which consists of a rod-shaped element lying external to the pronephros and constituting the greater portion of the dorsal zone (fig. 23, *s.sc*); b) the scapula, which lies just dorsal and anterior to the glenoid cavity and which, in the cartilaginous state, is continuous with the suprascapula (fig. 23, *sc*); c) the procoracoid lying immediately anterior and slightly ventral to the glenoid cavity (fig. 23, *p.cor*), and d) the coracoid, a relatively broad expanse of cartilage, constituting nearly the entire ventral zone of the girdle and reaching close to the mid-ventral line (fig. 7, *cor.* and fig. 23, *cor.*). The scapula, procoracoid, and coracoid are continuous proximally and enter into the formation of the glenoid cavity which receives the head of the humerus (fig. 23, *gc*).

Chondrification

Chondrification of the girdle proceeds gradually from the central portion towards the periphery. There are three centers, one for the scapula, one for the coracoid, and one for the procoracoid. The center for the scapula is first to appear. This is followed by the center for the coracoid and finally by the procoracoid center. This was found by Wiedersheim ('89) to be the case with Triton, Siredon, and Salamandra. The same observation was also made by Braus ('09) on Bombinator.

The union of these three centers completes the chondrification of the central portion of the girdle. The suprascapula has no separate center and chondrification of this element proceeds gradually from the region of the scapula in a dorsal direction.

While the central part of the girdle is chondrified before the first two digits of the fore limb are fully formed and before the elbow joint becomes visible, the dorsal portion of the suprascapula is not entirely chondrified until the fourth digit makes its appearance. The chondrification of the coracoid likewise proceeds gradually from its center towards the mid-ventral line.

These observations agree with those of Braus ('09) on Bombinator. In this form there are no chondrification centers for the suprascapula and the epicoracoid. The epicoracoid of Bombinator is homologous with the ventral portion of the coracoid in Amblystoma, which, as has been pointed out, chondrifies gradually from the proximal part towards the periphery.

The cartilage center for the humerus appears somewhat earlier than do those for the girdle. Considering the Amphibia as a whole, it can be said that in most cases this is true (Wiedersheim, '89, '90, '92; Lignitz, '97, and Braus, '09). From Strasser's ('79) description of Triton, one would assume, however, that in this form initial chondrification of the humerus and the girdle takes place simultaneously.

The centers for the ulna and radius appear slightly later than those for the girdle, but they are completely chondrified before chondrification of the suprascapula and the coracoid have been completed.

The greater part of the girdle remains cartilaginous throughout life, but the entire scapula and those portions of the procoracoid and coracoid which enter into the formation of the glenoid cavity become ossified. The cartilaginous suprascapula which, in the larva, is a long slender rod-shaped structure, elongates in an antero-posterior direction so as to become a broad flat plate. The procoracoid grows out in an antero-ventral direction and becomes a structure very similar in shape to the procoracoid of Necturus. The coracoid, which comprises the greater part of the ventral zone of the girdle, is a large flat rounded plate of cartilage lying ventral and posterior to the procoracoid. The two coracoids overlap in the mid-ventral line. The shape of the ventral portion of the adult girdle is very similar to that figured by Fürbringer ('73) for Salamandra maculata.

No description of the shoulder muscles of Amblystoma could be found in the literature. The musculature, however, so far as has been studied, closely resembles that of Salamandra maculata, a European tailed Amphibian described by Fürbringer (op. cit.). In referring to the musculature, Fürbringer's nomenclature will be employed.

3. EXPERIMENTAL

The experiments were carried out upon embryos in two different stages: a) the so-called tail-bud stage, and b) the stage of open medullary folds.

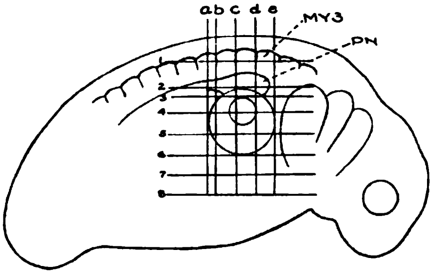


Fig. 1 Camera-lucida drawing of an embryo of Amblystoma in the tail-bud stage. The larger of the two circles represents the typical limb disc. × 15. *pn,* = pronephros.

1. EXPERIMENTS ON EMBRYOS IN THE TAIL-BUD STAGE (STAGE 29)

A. Extirpation experiments

1. Removal of the suprascapula rudiment. As has already been pointed out (Harrison, '15), the fore-limb rudiment of an embryo in the tail-bud stage consists of a somatopleural thickening just ventral to the pronephros, centering in the region of the fourth myotome and extending over into that of the third and fifth. The formation of a suprascapula following the extirpation of the limb rudiment in this stage shows that its rudiment is not included with the limb mesoderm. Although there is no *visible* suprascapula rudiment, nevertheless, extirpation of the region a–e × 1–3 (text fig. 1), including the outer or cutis layer of the

TABLE 1

Showing the results following the removal of the area a–e × 1–3 (text fig. 1) including the outer cell layer of the somites and the pronephros

INDIVIDUAL	AGE DAYS AFTER OPERATION	CONDITION OF THE GIRDLE AND THE EXTREMITY				
		Suprascapula	Scapula	Procoracoid	Coracoid	Humerus
E 8......	26	absent	present	present	present	present
Ba 10....	24	absent*	present	present	present	present
R 4......	26	absent	present	present	present	present
E 6......	26	absent	present	present	present	present

* Only dorsal half wanting.

ventral halves of the somites and the pronephros, results in the formation of a girdle without a suprascapula (fig. 24 and table 1). The removal of this area has practically no effect on the development of the extremity itself and of the remainder of the girdle. Abnormalities in the limb sometimes occur if its rudiment is disturbed to any great extent during the operation.

2. Removal of the suprascapula rudiment and of the myotomes. This type of experiment consisted in the entire removal of the third, fourth, and fifth somites and the pronephros. In these experiments the wound was usually covered with ectoderm taken from a second embryo, since complete exposure of the notochord and the medullary tube frequently results in a disintegration of the embryo. Removal of the entire somites produces the same effect on the girdle as that described for the first type of experiment, viz., the formation of a girdle without a suprascapula (figs. 8 and 25 and table 2). In the absence of the somites, the limb and the girdle undergo a dorsal shifting (fig. 8). This is apparently due to a release of pressure from that direction.

Complete removal of the third, fourth, and fifth somites, while leading to no defects in the limb musculature, brings about marked deficiencies in the ventro-lateral musculature, observations corroborating those previously made by Miss Byrnes ('98) and Lewis ('10). Miss Byrnes (op. cit.) produced the first experimental evidence to show that the musculature of the anterior limb of Amblystoma develops in situ and that it is in no way derived from the myotomes or their ventral processes.

TABLE 2

Showing the results following complete removal of the third, fourth, and fifth somites and the pronephros

INDIVIDUAL	AGE DAYS AFTER OPERATION	CONDITION OF THE GIRDLE AND THE EXTREMITY				
		Suprascapula	Scapula	Procoracoid	Coracoid	Humerus
Ba 13....	23	absent	present	present	present	present
Ba 11....	23	absent	present	present	present	undifferentiated
Ex 1.....	26	absent	present	present	present	absent
E 7......	27	dorsal half absent	present	present	present	present

The absence of the limb in case Ex 1 is no doubt due to the destruction of its rudiment in the removal of the pronephros.

Lewis (op. cit.), however, in addition to demonstrating this same fact, showed that definite defects in the ventro-lateral musculature follow the removal of the myotomes of the limb region.

An examination of figure 8 will show that, in this case, there is complete absence of the ventro-lateral musculature on the operated side. This is, however, not always the case, for in others in which these same myotomes were completely excised, the ventrolateral musculature is partly filled in by a compensatory elongation of the derivatives of intact myotomes. This same observation was also made by Lewis.

In the absence of the suprascapula, the m. dorsalis scapulae, which normally runs from the proximal end of the humerus over the external surface of the suprascapula (fig. 7, *m.dsc*), now attaches to the scapula (fig. 8).

It is essential, in order to remove successfully the suprascapula rudiment to include the pronephros. Its removal facilitates cleaning of the wound and thus affects indirectly the results of the experiment even though it exerts no direct influence. This applies as well to removal of the limb bud as a whole (Harrison, '15).

Since removal of the outer portions of the third, fourth, and fifth somites suppresses development of the suprascapula and since there is no visible rudiment for this element at the time of the operation, the experiments show that the suprascapula is derived from tissue other than that which gives rise to the limb and the remainder of the girdle. Sections of older embryos show that dorsal to the pronephros scattered mesenchyme cells gradually appear and become continuous, external to the pronephros, with the limb-forming cells. It is evident that this mesenchyme, which later forms the suprascapula, is segregated from the outer or cutis layer of the somites in this region and that the suprascapula is formed in situ. Such a conclusion is strengthened by the fact that after complete removal of the limb rudiment and the pronephros, the suprascapula develops in its normal place, provided the third, fourth, and fifth somites are undisturbed.

Not only do these experiments show that the suprascapula is already determined at the time of the operation and that it is formed in situ, but they demonstrate as well the impotency of the unremoved girdle tissue to replace the missing part.

3. Removal of the dorsal zone rudiment of the girdle and the limb mesoderm. This series of experiments consisted of the removal of the area a–e × 1–5 (text fig. 1). This included the outer portion of the ventral halves of the somites, the pronephros, and the limb mesoderm. The wounds were cleaned and covered. The removal of this area suppresses development of the suprascapula, scapula, and the free extremity, and only the ventral half of the girdle develops, no glenoid cavity being formed in any of these cases (figs. 9 and 26 and table 3). The formation in situ of the procoracoid and coracoid is evidence that they, too, are already determined at the time of the operation and are not dependent for differentiation on the remainder of the girdle and the limb, the rudiments of which were removed in this type of experiment. Although the myotomes proper were left intact, several larvae showed slight defects in the ventro-lateral musculature. This is no doubt due to a partial injury of the ventral portions which furnish the muscle buds.

The rudiments of practically all the shoulder musculature are included in these extirpations. In one case a few partially developed muscle fibers were present just external to the unremoved girdle elements. They probably represent the m. supra-

TABLE 3

Showing the results following the removal of the area a–e × 1–5 (text fig. 1) including the outer portion of the somites, the pronephros, and the limb mesoderm

INDIVIDUAL	AGE	CONDITION OF THE GIRDLE AND THE LIMB				
		Suprascapula	Scapula	Procoracoid	Coracoid	Humerus
	days					
R 6......	26	absent	absent	present	present	absent
E 2......	26	absent	absent	present	present	absent
R 2......	26	dorsal portion present	absent	present	present	absent
E 10.....	28	absent	absent	present	present	absent
Ex 3.....	28	absent	absent	present	present	absent
R 1......	26	small nodule of cartilage	absent	present	present	absent

Incomplete absence of the suprascapula in cases R 1 and R 2 is apparently due to imperfect removal of the rudiment.

coracoideus, a muscle which normally runs from the proximal end of the humerus to the external surface of the coracoid (fig. 7, *m.spc*).

4. Limb-bud extirpations. The effects of the removal of a typical limb disc on the girdle (text fig. 1) are in accord with those described by Harrison ('18). In individual H 2 sectioned twenty-two days after the operation, only the suprascapula, a very small procoracoid, and the coracoid were present (fig. 10 and table 4). In another, H 5, only the dorsal part of the suprascapula was present in addition to a fragmentary procoracoid and the coracoid (fig. 27 and table 4). In these cases the pronephros was removed with the limb bud, and the incompleteness of the suprascapula in the second case is no doubt due to a partial destruction of its rudiment in the removal of the pronephros.

Since no limbs developed in experiment 3 after the removal of the area a–e × 1–5, it is obvious that the ventral portion of a typical limb disc (Harrison, '15 and '18) which is shown in text figure 1 contains only girdle-forming cells.

The formation of only a portion of the ventral zone of the

TABLE 4

Showing the effects on the girdle of removal of the limb disc

INDIVIDUAL	AGE	CONDITION OF THE GIRDLE				
		Suprascapula	Scapula	Procoracoid	Coracoid	Shoulder joint
	days					
H 2......	20	present	absent	present	present	absent
H 5......	20	present*	absent	fragmentary	present	absent

* Dorsal half only.

girdle following a typical limb-bud extirpation not only indicates that part of its rudiment is removed with the limb bud, but that the part which is unremoved is already determined at the time of the operation. Further, when a limb bud is transplanted to a heterotopic position, the development of a girdle with a ventral zone of reduced size (fig. 28) also serves to indicate that only a portion of the rudiment is transferred with the limb cells.

If localization of the cells which are to form the ventral zone of the girdle is complete at the time of the operation and they can be successfully extirpated, then only the dorsal zone should develop following their excision. A series of experiments were therefore performed to test the validity of this hypothesis.

5. *Removal of the rudiment of the ventral zone of the girdle.* It is obvious, in the light of experiments previously described (A 3 and A 4), that, in attempts to extirpate the entire rudiment of the ventral zone, the excision must include not only a portion of the limb bud, but tissue lying still more ventral than this area. In these experiments the mesoderm within the area a–e × 4–8 was excised and the wounds cleaned and covered. While in seventy-five per cent of the cases normal limbs developed following excision of this area, it was found that, in a number of larvae sectioned twenty days after the operation, the ventral zone of the girdle was entirely wanting while the dorsal zone had developed normally (figs. 11, 12, and 29 and table 5). Several cases similar to the one figured, in which the entire rudiment of the ventral zone had been removed, not only lacked the procoracoid and coracoid elements, but the shoulder joint as well.

TABLE 5

Showing the results following the removal of the mesoderm within the area a–e × 4–8 (text fig. 1)

INDIVIDUAL	AGE	CONDITION OF THE GIRDLE AND THE EXTREMITY				
		Supra-scapula	Scapula	Procoracoid	Coracoid	Humerus
	days					
Ext. Gr. C. 4.	20	present	present	absent	rudimentary	present
Ext. Gr. C. 9.	20	present	present	absent	rudimentary	present
Ext. Gr. C. 13.	20	present	present	absent	absent	present
Ext. Gr. C. 19.	20	present	present	absent	absent	present
Ext. Gr. C. 20.	20	present	present	absent	absent	present

The development of a rudimentary coracoid in cases 4 and 9 is no doubt due to incomplete removal of the ventral zone rudiment.

The dorsal zone in these cases ended blindly at the point where the humerus began and was ankylosed with it. Shoulder movements in most of the cases were very slight or entirely wanting. In complete absence of the ventral zone, the procoraco-humeral and supracoracoideus muscles were also lacking, their rudiments having been removed with those of the cartilaginous elements. The pectoral muscles, however, were present and followed their normal course.

In other cases following the extirpation of this region the function of the limbs was more normal. It was found when these larvae were sectioned that, in addition to an imperfectly formed glenoid cavity, a small process of cartilage extended ventral to the proximal end of the humerus which could be identified as a very rudimentary coracoid. A short supracoracoideus muscle connected this fragmentary coracoid with the humerus. No procoracoid element could be distinguished.

The development of the rudimentary coracoid in these cases is evidence of incomplete removal of the entire rudiment of the ventral zone. Such cases, while they must be regarded in one sense as unsuccessful, have nevertheless an important bearing on the question of totipotency of the girdle-forming tissue. If the unremoved cells which formed this rudimentary coracoid were equipotential, then restitution of the entire ventral zone would be expected. This not being the case, it must be said that what

TABLE 6

Showing the results following removal of the mesoderm within the area a–e × 3–8 (text fig. 1)

INDIVIDUAL	AGE	CONDITION OF THE GIRDLE AND THE EXTREMITY				
		Supra-scapula	Scapula	Procoracoid	Coracoid	Humerus
	days					
Ext. Gr. C. 20	20	present	absent	absent	absent*	absent
Ext. Gr. C. 25	20	present	absent	absent	absent	absent

has been formed is an expression of the potency of these cells to form only that for which they are determined at the time of the operation. The rudimentary coracoid in these cases is smaller than that which developed from the tissue carried along with the limb cells in a typical limb-bud transplantation, but more coracoid-forming tissue is present in the latter case.

It is seen by referring to figure 1 that in the extirpation of the area a–e × 4–8 slightly more than the ventral half of the typical limb disc is included, yet fifteen out of twenty such cases developed normal limbs. This high percentage of normal limbs from only the more dorsal portion of the rudiment affords additional evidence of the true equipotentiality of the limb system.

6. *Removal of the ventral zone rudiment of the girdle and the entire limb rudiment.* This type of experiment consisted in the removal of the mesoderm within the area a–e × 3–8 (text fig. 1). All the wounds were cleaned and covered. The part removed included not only the limb mesoderm, but the rudiments of all parts of the girdle except the suprascapula. The results of these experiments are shown in figures 13 and 30 and in table 6.

The development in these cases of the suprascapula in the absence of the limb and all other parts of the girdle lends additional evidence of the independence of this element in its development, and shows further that it develops from a separate rudiment (Exp. A1, A2, and tables 1 and 2).

Braus was not able to say definitely whether or not in Bombinator the suprascapula had a separate rudiment. He was able to recognize two thickenings in the dorsal zone (Braus, '09, p. 175, fig. 5, *scap.*, and fig. 7, *Z*) which, however, were not sharply marked off from each other, thus suggesting the possibility of their being a single anlage.

While more or less definite defects in the girdle have resulted from the extirpation of regional areas hitherto designated, the girdle defects following the removal of either the anterior or posterior half of the limb disc have not been so definite nor constant in character. The few specimens used in this study were given over by Dr. Harrison, who removed from the embryos the anterior and posterior halves of the limb disc in connection with his experimental study of the equipotentiality of the limb.

Three cases were studied in which the anterior half of the disc had been removed. In cases Rem. E. 22 and 23, preserved forty-six and eighteen days, respectively, after the operation and in each of which the limb had developed normally, the only defect in the girdle consisted in the absence of the procoracoid. In case H. R. E. 11—(twenty-six days) in which the limb consisted of nothing more than a nodule of undifferentiated mesenchyme cells, there was complete absence of the girdle. This case bears no significance with regard to localization, since ordinarily after the removal of the *entire* limb disc, the suprascapula, procoracoid, and the coracoid develop (Exp. A 4 and table 4). The only point of interest that this case offered was the fact that in the absence of skeletal differentiation, two of the shoulder muscles were differentiated, these being the trapezius and the pectoral.

Three cases were studied in which the posterior half of the limb disc had been removed. Case Rem. E. 12 (twenty-six days) with normally developed extremity showed considerable defects in the ventral zone of the girdle. The procoracoid was entirely wanting, while the coracoid was greatly reduced in size. The dorsal zone was normal. In case H. R. E. 12—(twenty-six days) with defective hand, there was no procoracoid. The coracoid, while being normal in length, was somewhat reduced in width. The dorsal zone was also poorly developed. In H. R. E. 9—(twenty-six days) in which no limb had developed, all parts of the girdle were present although somewhat atypical in shape. The most pronounced abnormality consisted of a distinct exchondrosis from the posterior border of the suprascapula.

Reviewing these few cases as a whole, we see that they showed no *consistent* defects, yet it is hardly to be expected that the removal of either the anterior or the posterior half of the limb disc would bring about any marked deficiencies in the girdle, since only a very small portion of the girdle rudiment can be involved in either case. Even after the removal of the entire limb disc, the suprascapula, procoracoid, and the coracoid develop in their normal position, while only the scapula and the glenoid portion of the girdle are wanting. It is quite possible that the excision of larger anterior or posterior areas would produce more pronounced and definite defects.

B. Transplantation experiments

The development of a limb in the absence of either the dorsal or the ventral half of the girdle suggested the possibility of its development in the absence of the entire girdle. Experiments were therefore made to test this possibility.

1. Transplantation of small areas of cells from the dorsal half of the limb disc. The area of mesoderm transplanted in these experiments is shown in text figure 1 by the smaller of the two circles. In five cases out of fifteen such transplants the tissue was resorbed. The other ten developed into limbs which showed more or less abnormality, consisting chiefly of reduplicated hands. Most of the limbs were atrophic and all lacked function. It was found when these larvae were sectioned twenty days after the operation that only a very fragmentary piece of the girdle was present, which from its relation to the humerus must be regarded as a rudimentary coracoid (fig. 14, *cor.* and fig. 31, *cor*). This was connected with the humerus by a very short supracoracoideus muscle.

The almost complete absence of any girdle in these cases demonstrates that the region from which the transplanted tissue was taken is relatively free from cells which have the potency to form a girdle. When a larger area is transplanted, such as is indicated by the larger of the two circles in text figure 1, a considerable portion of the girdle-forming cells is included so that a girdle of the size shown in figure 28 develops at the place of implantation.

2. Transplantation of the ventral zone rudiment of the girdle and the ventral portion of the limb mesoderm. It has been shown in table 5 that extirpation of the mesoderm within the area a–e × 4–8 resulted in the formation of a girdle without a ventral zone (fig. 29). In this excision the ventral portion of the limb rudiment was included. This same area was transplanted in order to ascertain whether it would develop into a limb and a girdle consisting of only the ventral zone. Fifteen such transplantations were made. All developed limbs, of which ten showed reduplications of the hand; four were abortive and one normal. Three such cases were sectioned twenty-two days after the operation. The sections showed that only the ventral zone of the girdle was present with the limb (figs. 15 and 32). The limbs which resulted from these transplants developed from only a portion of the limb rudiment, showing thereby the equipotentiality of this system. The failure of the dorsal zone of the girdle to develop offers additional evidence to show the non-equipotentiality of the girdle rudiment.

3. Transplantation of the dorsal zone rudiment of the girdle and the dorsal portion of the limb mesoderm. This series of experiments consisted of the transplantation of the area a–e × 1–4 (text fig. 1) including the ventral halves of the somites, the pronephros, and the dorsal segment of the limb mesoderm. Ten such transplants gave rise to limbs, eight of which showed either reduplications of the hand alone or of both the forearm and hand. An examination of the girdles which had developed in these experiments shows that they are composed almost entirely of a dorsal zone (figs. 16 and 33), which, while somewhat smaller than the dorsal zone of a normal girdle, are nevertheless complete with respect to their elements, viz., suprascapula and scapula. The presence of a very small portion of the ventral zone in the girdle shown in figure 33 is no doubt due to the inclusion in the transplant of some of the cells which are to form the ventral zone, yet the failure of these cells to form a complete ventral zone shows again their lack of equipotentiality for this structure.

Experiments thus far described were made upon embryos at about the time of the appearance of the tail bud when the limb tissue is in the form of a definite limb bud. The relation of the girdle rudiment to that of the limb has been shown in these experiments. They have further served as a means of showing in two different ways—extirpation and transplantation—that not only is the girdle rudiment already determined at this stage, but that the rudiments of the separate components have already become localized.

A further discussion of these experiments will be taken up after the experiments in part 2 have been considered.

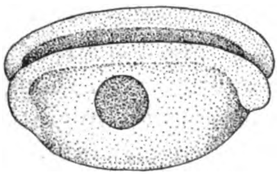
2. LIMB EXPERIMENTS ON EMBRYOS IN THE STAGE OF HIGH MEDULLARY FOLDS (STAGE 18) BEARING UPON THE DEVELOPMENT OF THE SHOULDER GIRDLE

A. Extirpation experiments

While the limb rudiment is present as a definite somatopleural thickening in the embryos used for all experiments hitherto described, its definiteness is gradually lost as we study successively earlier stages. In the stage of open medullary folds, no somatopleural thickening is present and the axial and the lateral mesoderm have not yet been separated. Therefore at this stage the limb rudiment is present as a region of mesoderm without visible local characteristics. Embryos at this stage also lack the surface markings, viz., somites and pronephros, which indicate the position of the limb rudiment in the older stage and which are used as landmarks in experimentation. Consequently, it is impossible to make *exact* determinations of the area of limb-forming cells.

Location of the limb mesoderm in the absence of surface markings was determined by a fairly accurate method, already described by the author (Detwiler, '17), a brief description of which may be repeated here.

After removing the embryos from the capsule a circular cut was made just posterior to an imaginary line passing dorsoventrally midway through the embryo. The ectoderm and mesoderm were removed. The wound, after being cleaned from all free mesoderm cells, was then covered over by a circular piece of ectoderm, cut to fit the excision, taken from an embryo which had been properly stained in an aqueous solution of Nile blue sulphate. In about one-half hour this stained disc of ectoderm had healed in and its size and position was then indicated by a camera drawing (text fig. 2). The heavily stippled area represents the circular piece of transplanted blue ectoderm, which is in marked contrast to the brownish-yellow ectoderm of the host.



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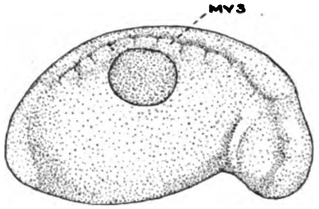

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Fig. 2 Camera drawing of an embryo (E. Tr. Ext. 12) in the stage of high medullary folds. The heavily stippled area represents a circular piece of ectoderm taken from an embryo previously stained in a solution of Nile blue sulphate. This occupies the wound resulting from the extirpation of the limb mesoderm. × 10.

Fig. 3 Camera drawing of the same embryo (E. Tr. Ext. 12) shown in figure 2 thirty hours later, showing the position of the inserted stained disc of ectoderm with reference to the somites. × 10.

The position of this inserted piece of ectoderm, when development had proceeded so that the somites had become visible, served to indicate whether or not the limb rudiment had been removed. If the stained disc eventually came to lie just ventral to the third, fourth, and fifth somites (text fig. 3), then it could be said with more or less certainty that the limb rudiments had been removed. When the extirpated area was taken from a more posterior position than that shown in text figure 2, the stained disc which covered the wound came to lie ventral to the fourth, fifth, and sixth or the fifth, sixth, and seventh somites. This indicated that only a portion of the limb rudiment had been removed and regeneration could be predicted.

The position of the stained disc is not always an absolute indicator of the region of the excised mesoderm, since it may be changed somewhat by ectodermal shifting. There is, however, very little shifting in the immediate limb region. The more pronounced ectodermal shifting, which does not usually occur until a somewhat later stage, takes place dorsal to the limb region and proceeds in a dorso-posterior direction; the general character and direction of the movement being similar to that observed in frog embryos by Harrison ('03) in his experiments on the development of the lateral line.

The migrating ectoderm, however, occasionally involves the dorsal portion of the stained area which then loses its circular shape and lengthens out dorso-posteriorally. Even though the stained disc is not involved in the ectodermal migration, it gradually becomes larger through continued cell division and the stain, now being distributed over a larger area, gradually loses its intensity.

It is impossible to excise equal areas in all cases and a certain degree of variation is to be expected. Measurements of the antero-posterior and dorso-ventral diameters of the inserted disc, after healing, were taken in many cases. These, of course, represent approximately the diameters of the excised piece. The size of this area was compared with that of a typical limb bud, which, in an embryo of the tail-bud stage has a diameter of three and one-half somites, an actual measurement of 0.93 mm. The diameters of the discs extirpated from the embryos in the neural fold stage varied between 0.66 and 0.93 mm.

Harrison ('15 and '18) showed in a series of extirpation experiments on embryos in the tail-bud stage that no regeneration occurred from wounds the width of three and one-half somites in diameter, provided they had been cleaned and covered, but when they were only three somites in diameter 33 per cent regeneration occurred. In most of the experiments on the neural-fold stage the average diameter of the excised areas was equal to the width of from three to three and one-half somites, or 0.73 to 0.93 mm. The results of the simple extirpations are given in table 7.

Although slight variations in the position from which these

TABLE 7

Showing the effect of removal of the limb mesoderm from embryos in the stage of high medullary folds

SIZE OF WOUND, DIAMETER	WOUNDS COVERED		WOUNDS NOT COVERED	
	Regenerated	Not regenerated	Regenerated	Not regenerated
mm.				
Not recorded	10	23	7	14
0.66	6	0		
0.73*	6	2		
0.80	1	3		
0.86	0	3		
0.93†	0	5		

* Equals the size of a three-somite wound.

† Equals the size of a three-and-one-half-somite wound.

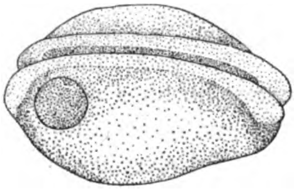
excised areas were taken undoubtedly occur because of the absence of definite surface markings, nevertheless, the results of the table show that, as the diameter of the excised area approaches the diameter of a three-and-one-half somite wound, less regeneration occurs.

While the limb mesoderm can be successfully extirpated from embryos of this stage certain technical difficulties are met with in the procedure. Although healing of the tissue is rapid, the embryos frequently disintegrate a day or two after the operation. It was found that a smaller number disintegrated if they were kept at a temperature of about 15°C. for twenty-four hours after the operation and then brought back to room temperature.

Watch glasses which were used in these operations were coated either with wax or paraffin in order to prevent the embryos from sticking to the bottom. If this is not done, they frequently adhere to the glass and the ectoderm is torn off in attempts to liberate them. Embryos should remain in these coated dishes for from twelve to twenty-four hours after the operation. They can then be transferred with safety to ordinary watch glasses.

B. Transplantation experiments

In the transplantation of the limb mesoderm the method of staining with Nile blue sulphate was also employed in a number of cases. Of two embryos which were selected, one was stained in an aqueous solution of the dye until the desired color had been attained. In the other embryo a circular wound was prepared, posterior to the region of the limb mesoderm, for the reception of the transplant. The limb mesoderm with the overlying ectoderm was then excised from the stained embryo (position indicated in text fig. 2) and transferred to the prepared wound (text fig. 4). It is seen from this figure that a transplant so placed occupies the flank region at the very posterior end of the embryo. When the embryo had developed so that the somites became visible, the position of the stained transplant with respect to the somites, as well as its relation to the normal limb region, could be ascertained (text fig. 5). Transplants which are placed at the position indicated in text figure 4 practically always come to lie ventral to the sixth, seventh, and eighth somites (text fig. 5), a position which is so near the normal limb region that conflicts frequently arise in the development of the two systems. The limbs which usually start separate development very often fuse together. In several cases only one limb developed from the two systems. In order to obviate these



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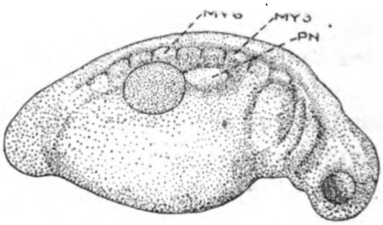

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Fig. 4 Camera drawing of an embryo in the stage of high medullary folds. The heavily stippled disc indicates the size and position of the limb mesoderm with the overlying ectoderm transplanted from an embryo previously stained in a solution of Nile blue sulphate. × 10.

Fig. 5 Camera drawing of the same embryo shown in figure 4 two days later, showing the relation of the transplanted area to the normal limb region. × 10.

TABLE 8

Showing the results of transplantation of the limb mesoderm from embryos in the stage of high medullary folds

ORIENTATION	NORMAL LIMBS	ABORTIVE	REDUPLICATIONS	FUSION WITH NORMAL LIMB	ABNORMAL SINGLE LIMBS	TRANSPLANT RESORBED
Homopleural dorso-dorsal	8	2	10	7	2	3
Homopleural dorso-ventral	2*	1	2	2	1	0
Heteropleural dorso-dorsal	2†	1	1	1	4	0

* Laterality reversed.

† Original laterality retained.

abnormalities, the transplants were later placed at the posterior end of older embryos. In such cases the transplant was of sufficient distance from the normal limb to allow independent development of the two systems.

The region in the stained embryo from which the transplant had been taken was in many cases covered with unstained ectoderm taken from a third embryo. This unstained insert now occupied the place from which the transplant had been taken and its position in the embryo, when the somites became visible, served to indicate whether the limb rudiment had been entirely or only in part transplanted.

The results of the transplantation experiments are given in table 8.

The posture of the normal limbs is in accord with the fundamental rules 1 and 2 underlying the determination of laterality (Harrison, '17). When the transplant is grafted on the same side from which it is taken (homopleural) and not inverted (dorso-dorsal), original laterality is retained (rule 1), but when inverted (dorso-ventral) a limb of opposite laterality results (rule 2). When transplanted to the side opposite to that from which it is taken (heteropleural) and not inverted, original laterality is retained (rule 1).

While the number of experiments is small, the results show that the fundamental asymmetry of the limb mesoderm is already determined in embryos with high medullary folds.

Just how early the limb rudiment is determined cannot be said at this time. It was successfully transplanted in several cases from embryos with low medullary folds, but no experiments have been made upon still younger embryos.

The region of mesoderm extirpated from the embryos in the stage of high medullary folds (text fig. 2) not only included the limb cells, but, in many cases, the entire girdle rudiment, so that there was complete absence of the girdle on the operated side (fig. 17). This figure is a section taken from the same embryo (E. Tr. Ext. 12) which is shown in text figure 2. The size of the region extirpated in this case corresponds to that of a three-somite wound in an embryo of the tail-bud stage, an extirpation which, in the later stage, removes only the central portion of the girdle rudiment so that isolated pieces of the girdle develop from the unremoved tissue (fig. 10). It is seen by comparing the two conditions resulting from extirpations of the same size that in the embryo with high medullary folds the girdle rudiment occupies a smaller area than in an embryo of the tail-bud stage.

The excised areas in the earlier stage always includes the rudiment of the pronephros. In the absence of the pronephros on the operated side a compensatory hypertrophy of this structure on the intact side is always found (fig. 17). This is in accord with Miss Howland's ('16) observations. Hypertrophy appears to be greater when the rudiment is removed from these early embryos than when the partially developed structure is removed from older embryos.

While in a number of these larvae, without the appendage, the entire girdle was lacking, in others a 'girdle' was found to be present (fig. 18). Although such girdles are present as continuous cartilaginous bands, nevertheless the central portion lacks those characteristics which typify the normal structure. This portion probably represents a hyperplastic growth of those parts which developed from the unremoved girdle cells which were located near the periphery of the wound. The completeness of these girdles therefore cannot be regarded as a manifestation of true restitution processes, for the completeness is only one of quantity and not of quality.

Since the region of mesoderm shown in text figure 2, when extirpated, frequently includes the entire girdle rudiment, it is obvious that when transplanted all or nearly all of the rudiment is included in the transplant.

When such transplantations were made it was found that the girdles which had developed at the place of implantation were almost as large as the normal girdles (fig. 20). Figure 19 represents a section through the normal girdle of individual D 29 preserved twenty-four days after the operation. Figure 20 shows a section through the implanted girdle. When these two girdles are compared and measured it is found that the implanted girdle is about three-fourths as large as the normal one. In individual D 41 preserved twenty-seven days after the operation the implanted girdle was also found to be three-fourths as large as the normal. In this case, as well as others, the implanted mesoderm was placed so near to the normal limb region that there was partial fusion of the two girdles. In individual D 23 preserved twenty-four days after the operation, the implanted girdle was larger than in all other operated cases studied, it being four-fifths the size of the normal. The dorsal zone was typical in shape, although the ventral zone was broader and not quite as long as that of the normal. There was also partial fusion of the two girdles.

The fact that double limbs frequently arise from a single transplanted rudiment has already been shown (Braus, '04 b, and Harrison, '07). When double limbs develop from transplants which are placed near the normal limb rudiment, the anterior of the two frequently unites with the normal limb to form a fused double limb, while the posterior member of the reduplication remains single. This condition was met with in individual D 23. Although the anterior member had fused with the normal limb, its shoulder joint was formed on the implanted girdle. A separate shoulder joint was formed some distance posterior and ventral to this for the reception of the posterior member of the reduplication. Other cases have shown that there may be only one shoulder joint for both members of a double limb. The formation of double limbs therefore does not mean the formation of a double girdle. In all cases studied a single girdle was formed. If the two members of a double limb are completely fused at the base there is only one shoulder joint; if they are separated at the base two joints may be formed on the same girdle.

According to Braus ('09, p. 283), when reduplicating limbs arise from a single transplanted rudiment of Bombinator, there may be a complete reduplication of the implanted girdle; or, in addition to the formation of one main girdle for the principal limb, there may be an accessory girdle for the supernumerary appendage (Braus, op. cit., p. 284, and pl. 16, fig. 5 a).

It is evident from the cases cited (D 23, 29, 41) in which girdles from three-fourths to four-fifths the size of the normal had developed, that all or nearly all of the rudiment must have been included in the transplant. Their being slightly smaller than the normal is probably due to the fact that they are placed in an abnormal environment where there is less space for differentiation than in the normal situation.

Girdles which develop from typical limb-bud transplantations in embryos of the tail-bud stage (text fig. 1) are never as large when compared with the normal as those which have just been described. When the girdle shown in figure 21, which is a typical result of such a transplant, is compared with the normal girdle of the same larva (fig. 22), it is found to be only one-third as large. The difference in size of the implanted girdles in the two cases becomes explicable when it is seen that in one case (fig. 21) only the central portion of the girdle rudiment is transplanted with the limb mesoderm, while in the other (fig. 20) all, or nearly all, of the rudiment is included.

4. DISCUSSION

In the embryos of Amblystoma individual portions of the shoulder-girdle rudiment are determined at the stage when the limb tissue is present as a definite somatopleural thickening (stage 29). Experimental evidence has been advanced to show that after extirpation of any definite portions of the rudiment only those components develop which are represented in unremoved tissue. Simple limb-bud extirpations (Exp. A 4, p. 507) demonstrate localization of only the more distal portions of the rudiment, since in the absence of the central portion, which is removed along with the limb bud, isolated elements (suprascapula and coracoid) of the girdle are formed. However, the formation of only the dorsal zone following complete removal of the rudiment of the ventral zone (Exp. A 5, p. 508) or just the opposite experiment (Exp. A 3, p. 506) offers proof that the central portion of the rudiment is also localized and that the extirpation of any given portion does not incite restitution processes in the unremoved parts.

The system at this stage may be regarded as a mosaic of which the various parts may develop into later known components without influence of neighboring portions. Braus ('09, p. 268) also called the shoulder-girdle system of Bombinator a mosaic, but only in its normal situation. According to him, when a portion of its rudiment is transplanted to a new environment, the mosaic is lost and the implanted tissue acquires the capacity of reversing differentiation which had already appeared in the anlage, so that it is now able to form anew an entire girdle. Accordingly, transplantation incites restitution, and the system, which in its normal environment is a mosaic, in the new environment becomes an equipotential one.

Although Braus found that the shoulder girdles which developed from implantations of only a portion of the rudiment were of reduced size (one-third to two-thirds the size of the normal), nevertheless, they were regarded by him as entire, just as complete individuals of reduced size are formed from isolated blastomeres in the cleavage stage of an egg or from fertilized egg fragments. While the girdles which had developed at the implanted place were formed in all cases from the central portion of the rudiment, since it is this portion which is included with the limb cells, Braus nevertheless questioned whether there was any greater inherent capacity on the part of any portion of the anlage over any other in the process of restitution. According to this idea, the entire structure might develop from either the dorsal or ventral parts of the rudiments, yet no experiments were made to test the validity of this hypothesis.

While Braus ('09, p. 27) claimed that this system in Bombinator is equipotential from stage 2, when the limb bud is very small, up to the time of chondrification of the humerus, it is clear from experiments described in this paper that such is not the case in Amblystoma.

It has been shown, that when only the ventral portion of the rudiment was transplanted with the limb cells, only the corresponding zone of the girdle developed (figs. 15 and 32), just as when the dorsal half of the rudiment was transplanted with the limb cells, it developed into a girdle which was composed almost entirely of a dorsal zone (figs. 16 and 33). The presence of a very small portion of the ventral zone in these cases is no doubt due to the inclusion in the transplant of some of the cells which are to form the ventral zone, since it is difficult to separate accurately the two halves of the rudiment. The failure, however, of these cells to form a complete ventral zone shows their lack of the potency to form this portion of the girdle.

Although the girdle rudiment of Amblystoma, when transplanted, may not be regarded as a perfect mosaic, since slightly more cartilage may form from portions of the rudiment than would have formed had they not been transplanted, yet it is obvious that it cannot be regarded as an equipotential system at the stage with which we are dealing. In a true equipotential system such as the limb rudiment, the extirpation of any portion does not lead to any definite defects in the components of the limb which develop from the unremoved portion of the rudiment. Even after extirpation of a half of the rudiment, normal limbs with all their cartilaginous and muscular elements may develop. The same thing is true when a half of the limb rudiment is transplanted. In this case two limbs of normal size and structure may develop, one from the transplant and the other from the unremoved half of the rudiment.

Definite defects in the girdle resulting from removal of certain portions of its rudiment, as well as the formation of girdles with only dorsal or ventral halves resulting from the transplantation of the corresponding portions of the rudiment, are sufficient evidence to show that the system cannot be regarded as being equipotential at the stage when the limb mesoderm is in the form of a definite limb bud.

Experiments upon embryos in the stage of open medullary folds show that the girdle rudiment occupied a smaller area than in embryos of the tail-bud stage and that it was frequently entirely removed along with the limb cells so that no girdle developed. In other cases where it was not entirely removed a complete cartilaginous band was formed which, upon superficial examination, resembled a normal girdle (fig. 18). This cannot be regarded necessarily as a complete girdle, however, for it is lacking in certain components which typify the normal structure. The central portion of a girdle of this type no doubt represents a hyperplastic growth of the more distal portions whose rudiments were not removed with the excised area, and the completeness of this structure must be regarded as one of quantity rather than one of quality. From the incompleteness of quality of a structure of this type, it is seen that even at thestage of open medullary folds the system is not capable of qualitative restitution. This is strengthened by the fact that in several cases the girdles which had developed from implanted mesoderm at this stage were composed of only the ventral zone, indicating that only the corresponding portion of the rudiment was transplanted.

It has been shown that girdles which develop from transplanted areas in stage 18 were considerably larger than those which formed from areas of equal size in stage 29 (compare figs. 20 and 21). This condition might be used as a point in favor of equipotentiality of the system in the early stage, were it not known that in the former case all or nearly all of the rudiment was transplanted, while in the latter case only the central portion.

Braus ('09, p. 277) claimed that, between his stage 2, when the limb bud first becomes visible, and the stage when chondrification of the humerus begins, an area of transplanted mesoderm taken from a younger embryo did not develop into a girdle any larger than that which developed from the same sized area from an older embryo. As has already been shown, this is not the case in Amblystoma between the stage of open medullary folds and the tail-bud stage. These stages, however, are younger than any used by Braus.

Variations, therefore, in the size of the implanted girdle are to be regarded as an expression of a greater or less amount of girdle rudiment having been included with the transplanted limb mesoderm.

5. SUMMARY

1. The separate parts of the shoulder-girdle rudiment of Amblystoma punctatum are already determined at the stage when the limb rudiment is present as a definite thickening of the somatopleure (Stage 29).
2. The removal of a definite portion of the rudiment brings about a defect in that portion of the girdle corresponding to the part removed.
3. The extirpation of a given portion of the rudiment does not incite restitution processes in the unremoved parts. In the absence of the central part of the girdle the gap may be bridged over by a hyperplastic development of those elements which are formed from the unremoved portions of the rudiment.
4. The suprascapula develops 'in situ' from a separate rudiment, the extirpation of which has no effect on the development of the limb nor on the remainder of the girdle (Exp. A 1, fig. 24, and table 1).
5. Complete extirpation of the third, fourth, and fifth somites, while having no effect on the musculature of the limb, does, however, produce marked defects on the ventro-lateral musculature (Exp. A 2). These results corroborate those of Byrnes ('98) and Lewis ('10).
6. The central portion of the girdle rudiment is carried along in the transplantation of a typical limb bud (text fig. 1) and a girdle of about one-third the size of the normal develops at the place of implantation (fig. 21; cf. fig. 22).
7. It is possible to initiate the development of a limb in

almost complete absence of the shoulder girdle by transplanting a small area of cells (text fig. 1, smaller of the two circles) from the dorsal half of a typical limb disc (fig. 31). This area is practically free from girdle-forming cells.

8. Either the dorsal or ventral half of the girdle rudiment may be transplanted along with the corresponding portions of the limb rudiment. In such cases complete limbs develop from the transplanted portion of its rudiment, while only that portion of the girdle develops corresponding to the part of the rudiment which has been implanted (Exps. B 2, B 3, and figs. 32 and 33). These results demonstrate the non-equipotentiality of the girdle system.

9. The limb mesoderm is already determined in embryos in the stage of open medullary folds. The rudiment can be successfully extirpated and transplanted at this stage (tables 7 and 8).

10. In the absence of surface markings, the position of the limb mesoderm can be located quite accurately by covering the wound with ectoderm from an embryo, previously stained in a solution of Nile blue sulphate, and observing its relation to the later formed somites (text figs. 2 and 3).

11. When typical limb buds the width of three and one-half somites in diameter (text fig. 1) are extirpated from embryos in the tail-bud stage, only the central portion of the girdle rudiment is included (Exp. A 4, fig. 10); but areas of equal size when extirpated from embryos in the stage of high medullary folds (text fig. 2) frequently include the entire girdle rudiment (fig. 17).

12. Implanted girdles developing from areas of mesoderm, which are taken from embryos in the stage of open medullary folds, are considerably larger when compared with the normal than those which develop from areas of equal size taken from embryos in the tail-bud stage (cf. figs. 19, 20, 21 and 22).

13. When double limbs arise from a transplanted rudiment, only one girdle is formed, which may have one or two shoulder joints, depending on whether or not the two members of the reduplication are completely fused or partly separated at the base.

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PLATES

All of the figures in plates 1, 2, and 3 are microphotographs of transverse sections cut 10 μ in thickness and magnified 30 diameters. The larvae were fixed in sublimate-acetic killing fluid and the sections stained with Ehrlich's haematoxylin and Congo-red. All operations were made on the right side which, in the figures, appears as the *left*.

ABBREVIATIONS

cor, coracoid
g.c, glenoid cavity
hum, humerus
m.dsc, musc. dorsalis scapulae
m.spc, musc. supracoracoideus
my, myotome

p.cor, procoracoid
pn, pronephros
sc, scapula
s.sc, suprascapula
vlm, ventro-lateral musculature

PLATE 1

EXPLANATION OF FIGURES

Fig. 6 A section through the shoulder girdle of a normal larva twenty-five days after the closure of the medullary folds.

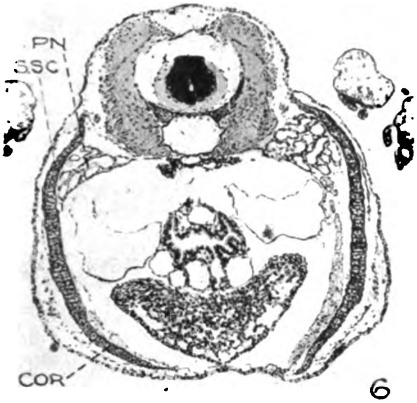
Fig. 7 A section through the glenoid cavity and the coracoid portion of the girdle of a normal larva.

Fig. 8 A section through the shoulder region of individual Ba. 13, showing the effect on the girdle of the entire removal of the third, fourth, and fifth somites.

Fig. 9 A section through the shoulder girdle of individual E. 2 developed in the absence of the rudiments of the suprascapula, scapula, and the limb (a–e × 1–5, text fig. 1).

Fig. 10 A section through the shoulder region of individual H. 2 showing the absence of the central part of the girdle, the rudiment of which is removed in the extirpation of a typical limb bud (text fig. 1).

Fig. 11 A section through the shoulder girdle of individual Ext. Gr. C. 19 which developed after the removal of the rudiments of the ventral zone (a–e × 4–8, text fig. 1).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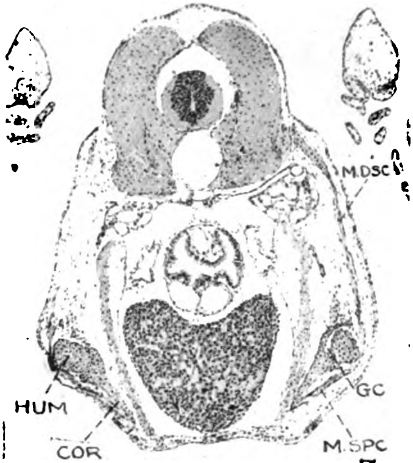
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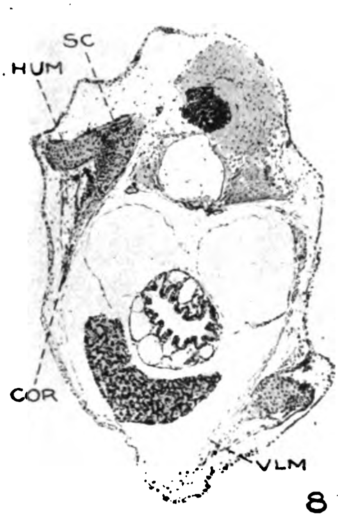
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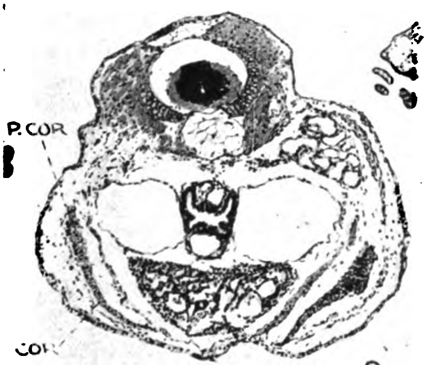
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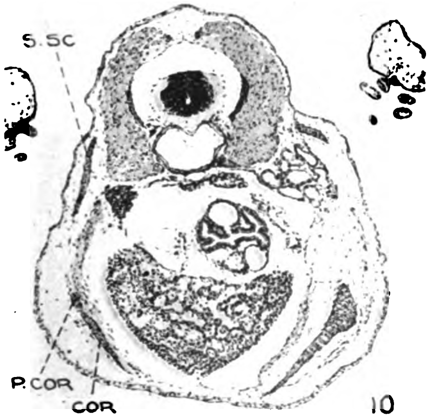
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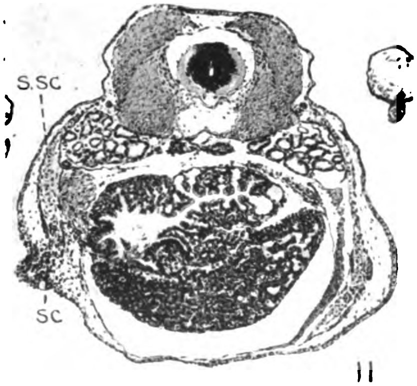
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PLATE 2

EXPLANATION OF FIGURES

Fig. 12 A section through the shoulder region of individual Ext. Gr. C. 19 slightly posterior to that shown in figure 11, showing the absence of the glenoid cavity and the ventral zone of the girdle (compare with fig. 7).

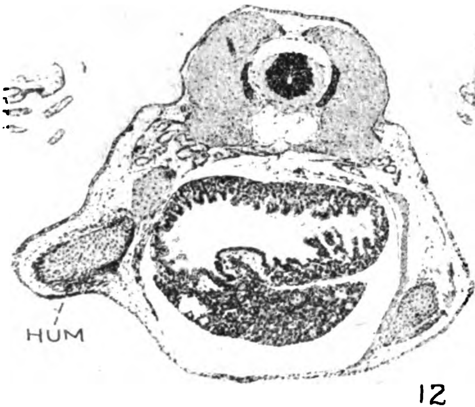
Fig. 13 A section through the shoulder region of individual Ext. Gr. C. 25, showing the development of the suprascapula after the rudiments of all other parts of the girdle had been removed (a–e × 3–8, text fig. 1).

Fig. 14 A section of individual S. B. 2, showing the presence of only a very fragmentary portion of the girdle which developed, along with the limb, from the transplantation of a small area of cells taken from the dorsal portion of the limb disc of another embryo (text fig. 1, smaller of the two circles).

Fig. 15 A section of individual Tr. 2/v 10, showing the girdle which developed in a heterotopic position from the transplantation, along with a portion of the limb mesoderm, of the ventral half of the girdle rudiment (a–e × 4–8, text fig. 1).

Fig. 16 A section of individual Tr. d/2 4, showing the shoulder girdle which developed in a heterotopic position from the transplantation of the dorsal half of the girdle rudiment along with a corresponding portion of the limb mesoderm (a–e × 1–4, text fig. 1).

Fig. 17 A section from the shoulder region of individual E. Tr. Ext. 12 showing complete absence of the girdle, the rudiment of which was removed along with the limb mesoderm from the embryo in the stage of open medullary folds (text fig. 2).



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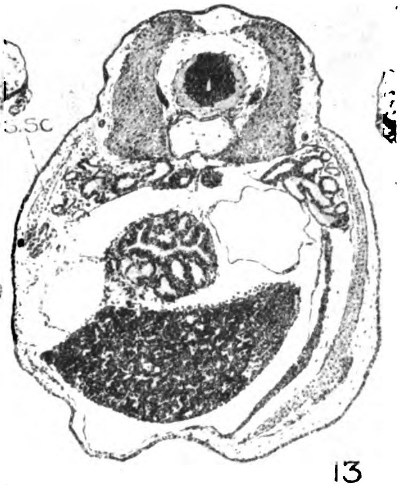

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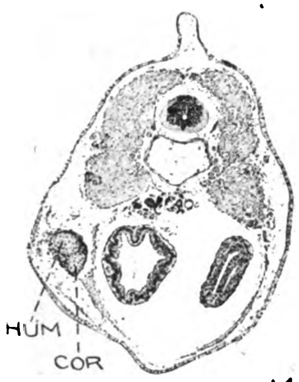

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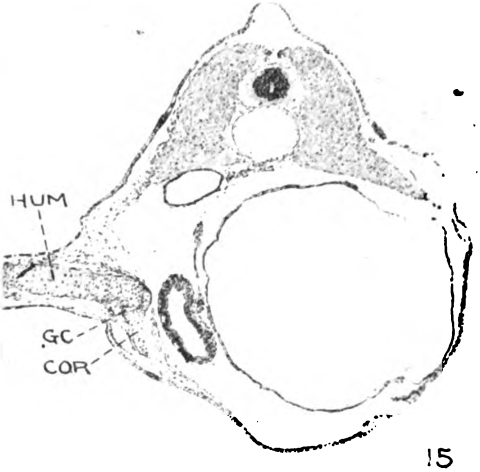

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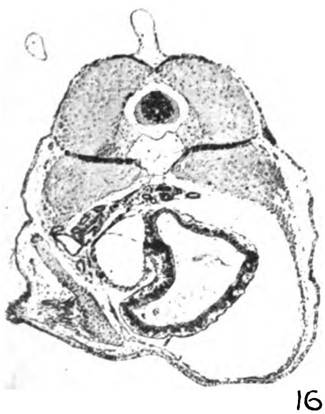

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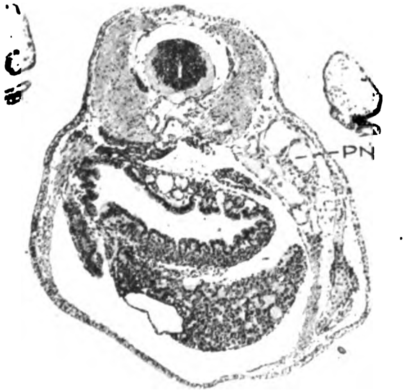

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PLATE 3

EXPLANATION OF FIGURES

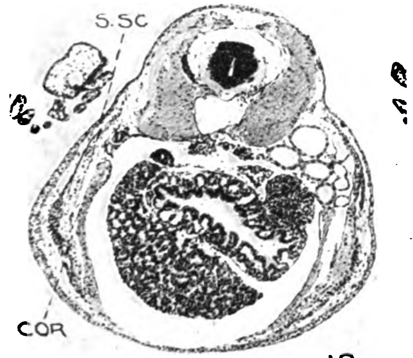
Fig. 18 A section through the girdle of individual Ca. 6 developed in the absence of the limb and the central portion of the girdle rudiment.

Fig. 19 A section through the normal girdle of individual D 29.

Fig. 20 A section from individual D 29 showing the girdle which developed in a heterotopic position from the implantation, along with the limb mesoderm, of the girdle rudiment from an embryo in the stage of open medullary folds.

Fig. 21 A section through the implanted girdle of individual Tr. Ext. 154 developed from the central portion of the rudiment which was transplanted, along with the limb rudiment from an embryo in the tail-bud stage.

Fig. 22 A section through the normal shoulder girdle of individual Tr. Ext. 154.



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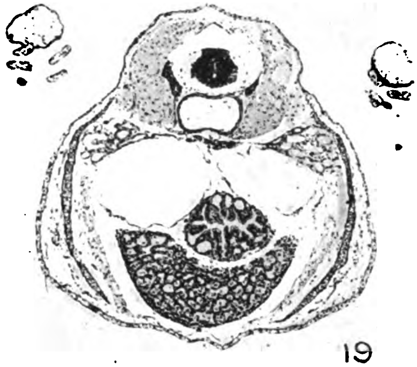

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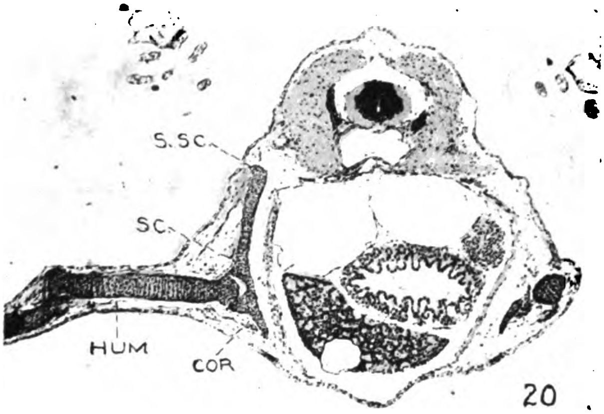

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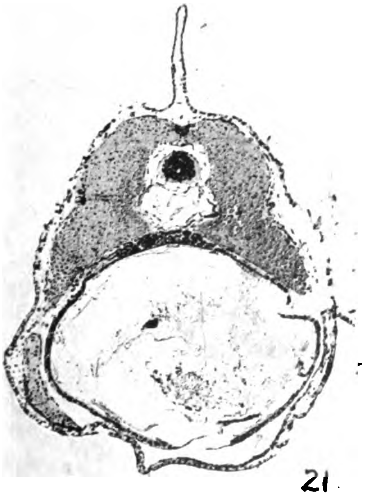

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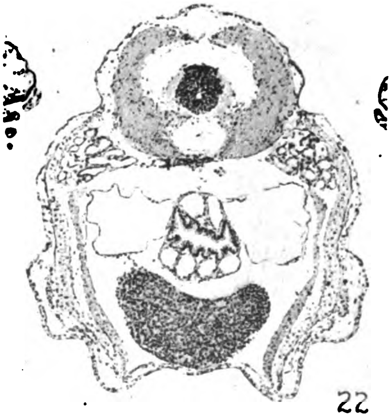

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PLATE 4

EXPLANATION OF FIGURES

All of the figures are photographs of blotting paper reconstruction models of the shoulder girdle. The models were made at a magnification of 100 diameters and reduced one-half for publication.

Fig. 23 Model of the left normal shoulder girdle of individual Ba. 13.

Fig. 24 Model of the right shoulder girdle of individual R. 4 which developed in the absence of the suprascapula rudiment.

Fig. 25 Model of the right shoulder girdle of individual Ba. 13 developed in the absence of the third, fourth, and fifth somites.

Fig. 26 Model of the right shoulder girdle of individual E. 2 developed in the absence of the rudiments of the suprascapula, scapula and the limb (compare with fig. 9).

Fig. 27 Model of the right shoulder girdle of individual H. 5 developed in the absence of the central portion of the rudiment which was removed along with the limb bud (compare with fig. 10).

Fig. 28 Model of the implanted girdle of individual Tr. Ext. 154 developed from the central portion of the rudiment which was transplanted along with a typical limb disc (compare with fig. 21).

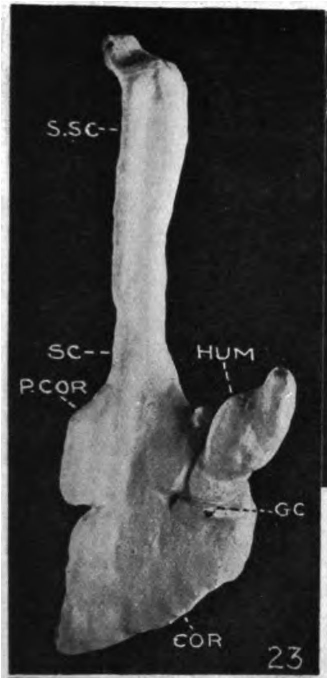
Fig. 29 Model of the right shoulder girdle of individual Ext. Gr. C. 13 developed in the absence of the rudiment of the ventral zone (compare with figs. 11 and 12).

Fig. 30 Model of the right suprascapula of individual Ext. Gr. C. 25 developed in the absence of the limb and the remainder of the girdle (compare with fig. 13).

Fig. 31 Model showing a very fragmentary girdle which developed, along with the limb, from the implantation of a small area of cells taken from the dorsal portion of the limb disc (compare with fig. 14).

Fig. 32 Model of an implanted girdle from individual Tr. 2/v 10 which developed in a heterotopic position from the transplantation of the ventral zone of the girdle rudiment (compare with fig. 15).

Fig. 33 Model of an implanted girdle from individual Tr. d/2 4 which developed from the transplantation, along with the dorsal portion of the limb mesoderm, of the dorsal zone of the girdle rudiment (compare with fig. 16).



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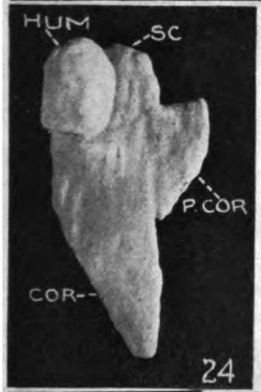

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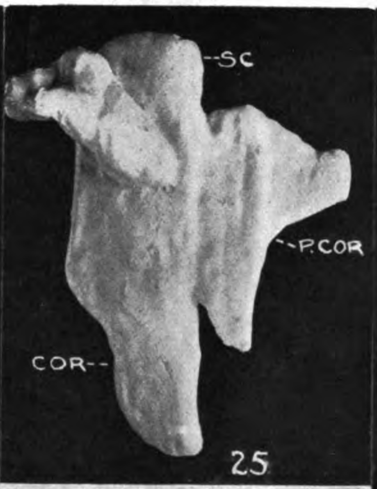

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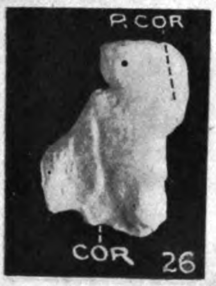

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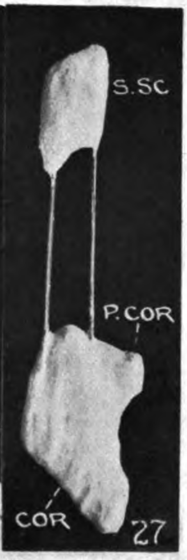

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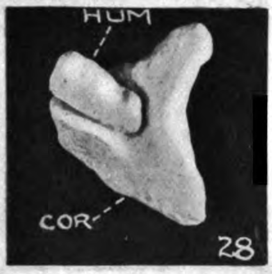

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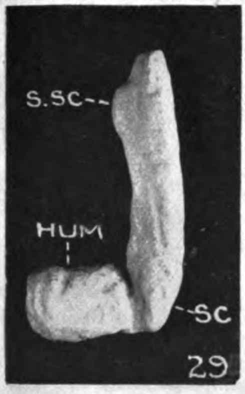

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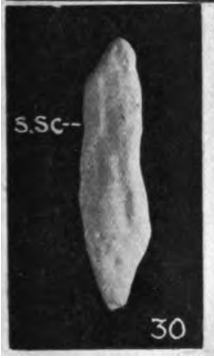

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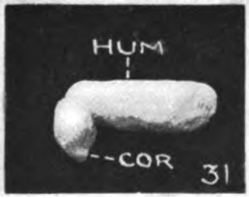

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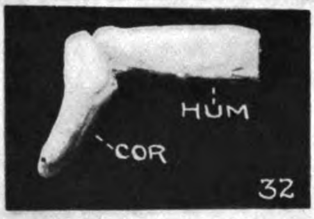

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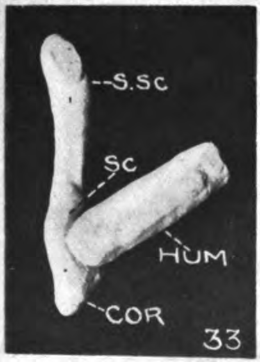

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1918, xv, pp. 96–98.

166 (1344)

Production of transplantable growth.

By **Rhoda Erdmann**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

After analyzing the known numerous theories on the origin of abnormal growth and especially of malignant growth, which have been devised either with or without reference to facts, it seems possible to concentrate the apparently necessary postulates for the successful origin of abnormal growth to the following conditions which cover all important theories on the subject. This presence of a cell group in an indifferent stage of growth, the dissociation of this cell group from its surrounding and its change from the indifferent stage of growth to a stage of proliferation, the inflammation of the connective tissue, neighboring this cell group, and a changed metabolism of the cells, in which this cell penetrates and which it destroys. Supposed these conditions are true to actual facts it must be possible to produce experimentally these factors either in various sequence or all at once and observe results.

I. Embryonic tissue of the chicken heart, after twelve days, incubation, was cultivated in a plasma medium for three days, and three 1 mm. square pieces implanted subcutaneously into a chicken. The implants produced after *ten days* small protuberances. Sections show cyst formation, caused by the isolation of the epidermis above the implants and *inflammatory* proliferation of the connective tissue of the host. Cyst formation, destruction of the feather follicles and accessory apparatus of the feather after previous isolation of the feather-forming complexes are visible. The implanted embryonic heart-muscle tissue which is surrounded by lymphocytes and newly formed connective tissue cells shows no considerable development. Protuberances produced in the same manner disappear after three weeks, as was to be expected.

II. The size of such a growth can be enlarged by a few injections of a *growth-promoting* substance, *i. e.*, tetheline, into the protuberance itself. The growth treated with tetheline is three times as big as its untreated neighbor. Sections show the implant, bordering on the unchanged skin, closely connected with it by newly formed tissue. The embryonic heart-muscle tissue particles are visible after 10 days. Newly formed connective tissue penetrates into the subcutaneous connective tissue of the host. The implant is vascularized by the blood vessels of the chicken. In the skin above the implant, the previously described disarrangement of the epidermal parts and the feather follicles has also taken place. But though these growths often reach the size of a hazelnut, they are finally resorbed.

III. The diet of the host, in which the prepared tissue particle was going to be implanted, was now changed from mixed diet to a vitamine-restricted diet. Chickens were fed with corn gluten meal and the same implantation performed. Breasts of chickens of the same age, but fed on different diet, showed a marked difference in the development of the breast muscles which are nearly atrophied in chickens on vitamine-restricted diet. Protuberance appeared after implantation in the same manner, as in birds fed with mixed food. After twenty-four days half of a protuberance was extracted and sectioned. The destruction processes in the skin are nearly finished, all feather follicles are destroyed, and it could be seen during the extraction that the tissue of the growth had penetrated into the underlying tissue of the host. The remaining half of the protuberance gradually disappeared and in its stead a tumor-like growth appeared. It was partially extracted eight weeks after the original implantation, the chicken dying three days after this operation. Horizontal and transverse sections show the penetration of fibrous connective tissue into the striped muscles, their compression and destruction, together with compressed blood vessels and blood sinuses. Where these processes are finished muscular tissue and fibrous connective tissue of surprising structure appear. The hard tumor shows macroscopically deep pockets in which we see the destroyed blood vessels and muscles; they seem to be filled with brown detritus. Part of this original growth could be successfully grafted on chickens on vitamine-restricted diet. The fourth generation after thirteen days' growth from February 12 to 25, 1918, showed the character of the original growth, especially the capacity to penetrate into the underlying tissues and to destroy them.

I will not omit to point out that the growth described here did not show bacterial contamination. The possible objection, that this growth which is very similar to those described by Rous,[1] 1910, *a* and *b*, and Rous and Lange,[2] 1913 (compare 1910$_a$, Fig. 5, 1910$_b$, Figs. 2 and 3, and 1913, Fig. 7) might be a "spontaneous growth," I will refute immediately by saying that the repetition of this series of experiments on a larger scale was successful. Only in two of twelve chickens, which had received (1) implantation of heart tissue, (2) tetheline injections into the implant, (3) vitamine-restricted diet, growths were produced, the other ten in which one or two different factors are lacking, showed no protuberance formation.

The produced growth, the genesis of which I have outlined, seems to be a connective tissue growth. It is infiltrable, destructive and transplantable. Further cytological investigation, especially the solution of the question, whether the implanted embryonic tissue cells are really forming by their descendants part of the growth, will be necessary to name this transplantable growth correctly.

A detailed description of this growth and two others, together with their descendants, will appear later.

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[1] *Rous, Jour. Exp. Medicina,* 1910, p. 285 and p. 397.

[2] Rous and Lange, *ibidem,* 1913, p. 651.

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EXPERIMENTS ON THE DEVELOPMENT OF THE FORE LIMB OF AMBLYSTOMA, A SELF-DIFFERENTIATING EQUIPOTENTIAL SYSTEM

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FORTY-FIVE FIGURES

Since Balfour's ('78) discovery of muscle buds in the developing fins of elasmobranchs the composite nature of the vertebrate limb has been emphasized. In the lower forms at least five different embryonal tissues are involved in its make-up, and these recur in part metamerically. Primarily there is a local thickening of the somatopleure which gives rise to the main bulk of the limb—the skeleton and other connective-tissue elements. This is covered over by ectoderm which in that region shows distinct modification. Into the mesenchymal mass derived from the somatopleure the muscle buds, blood vessels and nerves grow sooner or later from the trunk of the embryo.

Some years ago it was pointed out (Harrison, '95) that the various fins of the salmon form a distinct series as regards original visible complexity, beginning with the median fins where metameric muscle buds are clearly formed, as in the elasmobranchs, and ending with the pectoral fin, which receives no muscle buds. Here the proliferation of the somatopleure forms a blastema which gives rise to the muscles as well as to the skeletal elements.[1] As compared with the primitive elasmobranch fin, the pectoral fin is thus a more compact and unified organ at the time of its origin, and this is true in even higher degree for both extremities in the Amphibia. Probably also the limbs of the

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[1] Derjugin ('08) has described what purport to be muscle buds in the pectoral fins of Exocoetus volitans. In view of the differences in the fins of a single species, Salmo salar, it would not be strange, were specific differences of this kind to be found in the pectoral fin of various bony fishes.

Amniota, with possible exceptions among the Reptilia (van Bemmelen, '89, Mollier, '95) develop according to the latter type.

The first experimental work aiming at an analysis of these factors was done by Byrnes ('98 a), who found that the normal development of the posterior extremity in both Amblystoma and Rana is independent of the presence of the muscle plates of the limb region. More recently this same point has been demonstrated with greater precision by Lewis ('10), who has shown that while extirpation of muscle plates in the limb region of Amblystoma produces very definite defects in the lateral and ventral musculature, it has no effect upon the development of the limb itself.

It was found by Langnecker (Harrison, '04) that the development of the skeletal and muscular elements of the limbs of the frog would take place even when the nerves of the limb were excluded by operation on the embryo. This was afterwards confirmed by Braus ('05), using different methods of experimentation.[2]

There remain, then, as possible factors determining the differentiation of the limb bud from the body wall in general, *a*) the potencies of the ectoderm cells of the region; *b*) the potencies of the somatopleuric mesoderm cells, and *c*) the position of these elements with respect to the organism as a whole, or perhaps better with respect to its immediate surroundings, including, in the case of the fore limb, the pronephros and the branchial region,[3] which have close topographical relations to the limb rudiment. The blood vessels are not included in this enumeration, for while it is obvious that they are necessary for the nourishment and growth of the limb, no specific action on their part can be assumed. Braus ('03, '04, '09) found that the limb bud of the anuran larva constitutes a self-differentiating system which develops into a normal limb when transplanted to new and strange surroundings. This important discovery, which was confirmed independently by Banchi ('04, '05) and later by the present writer ('07) shows that once the development of the limb has started, its position in the organism as a whole does not affect its specific character.

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[2] Braus ('06 a) also showed that in the elasmobranch fin certain skeletal elements, the cartilaginous rays, would develop independently of the muscle buds.

[3] Braus has suggested ('06 b, p. 545), on the basis of experiments upon Rana embryos, that the gill region may exert a formative influence upon the regeneration of the fore limb, though no evidence is adduced to show whether this region has an influence upon the normal development of the limb.

The experiments taken up in the present paper, which deal with the fore limb of the urodele embryo, are designed to apply the tests of self-differentiation to a much earlier period of development. Considering for the present only those stages with closed medullary folds, the experiments show that the immediate organic environment of the limb rudiment, even before visible differentiation sets in, has no specific influence in determining that a limb shall develop, though it may affect the posture and laterality of the limb that does arise (Harrison, '15, '17). The essential process of differentiation, whereby the potency to form a fore limb becomes localized in certain cells of the body wall, must therefore be relegated to very early embryonic life.[4] It is further shown that in the stages under consideration this potency is localized in the mesoderm of the region involved, the ectoderm remaining indifferent.

The object of the experiments, aside from their purpose in further analyzing the factors taking part in the formation of the vertebrate limb, was to study certain relations which have a direct practical bearing upon the experiments on the laterality of limbs to be described later. An effort has been made to determine precisely the extent of the limb rudiment in the stages used for operation. In addition, the question of the equipotentiality of the elements composing the limb rudiment has been investigated both by defect experiments and by transplantation.

In interpreting defect experiments there is always a serious source of error in the occurrence of processes of regeneration or regulation, whereby elements which do not normally give rise to the part which has been removed may do so vicariously under the changed conditions. Such processes are known to occur in connection with experiments upon the limbs and must therefore be taken into consideration. For instance, Miss Byrnes ('98 b), working with Rana embryos, found that if the region in which the hind limb develops is destroyed, a limb nevertheless develops out of the tissues that move in from the periphery and cover the wound. Braus ('06 b) found, however, that in Bombinator, as well as in Rana, the hind-limb region is much more capable of this kind of regeneration than is the fore-limb region.[5] In the case of the latter, as Braus ('09) shows, it is only the shoulder girdle and some of the muscles that are formed after removal of the limb bud, unless the operation is done at a comparatively early stage, in which case regeneration of the whole extremity may take place. When the limb bud is itself transplanted it gives rise to a small shoulder girdle with some of the muscles in addition to the free appendage, so that in such an experiment certain muscles and skeletal elements develop only at the seat of origin of the bud, certain others develop only out of the transplanted bud itself, while again others develop in both places. The same has been found true in many cases in the course of the present work upon Amblystoma. Under certain conditions, however, the whole limb may be regenerated after removal of the bud, two complete limbs being thus derived from the single limb rudiment. The size of the extirpated region of body wall and the completeness with which all mesoderm cells within that region are removed have been found to be important factors in determining whether complete regeneration takes place or not.

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[4] Before the formation of the medullary folds according to Detwiler ('18).

In connection with the duplicated development of the shoulder girdle in its original position and at the seat of implantation, Braus has treated in full the question of mosaic development vs. equipotentiality.[6] He reaches the conclusion that in the Anura the shoulder girdle constitutes an equipotential restitution system, though in its original surroundings there is evidence of localization of materials before visible differentiation sets in. The results recorded in the present paper agree as to the main facts with those of Braus, though they leave uncertainty as to whether in Amblystoma there is complete qualitative restitution of the shoulder girdle in either place.[7] Whichever view may be accepted for the shoulder girdle, all of the evidence accumulated here goes to show that the free extremity, while self-differentiating, must be considered as an equipotential system in Driesch's sense.[8]

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[5] In R. esculenta, according to Braus ('09, footnote, p. 194), the power of regeneration is greater than in Bombinator.

[6] Braus, '09, p. 271, et seq.

MATERIAL, METHODS AND NORMAL DEVELOPMENT

All the experiments have been made upon embryos of Amblystoma punctatum in stages ranging between those shown in figures 1 and 3. This species offers great advantages over the anurans, which have been largely used in previous experiments upon the limbs. The fore-limb rudiment develops very early and rapidly, and may be located with precision shortly after the medullary folds close, before the embryo can move, and before blood vessels or nerves have developed. Experiments can, therefore, be carried out without anaesthesia, and the different tissues or embryonic organs can be easily separated from one another. The absence of a closed operculum, which in the anurans covers up the fore limb, is also a great advantage in that it permits the study of the progress of the experiment on the living individual, which may be examined from day to day without difficulty.

The technique of the surgery of the Amphibian embryo is now so well known that no general description is required here. The special operations used in the present investigation will be described as far as needed in the separate sections.

In the earliest stages at which operations were done the limb rudiment cannot be distinguished by any definite characteristic save its location. In the stage with beginning tail bud (fig. 1, stage 25[9]) the pronephric swelling becomes visible and the muscle

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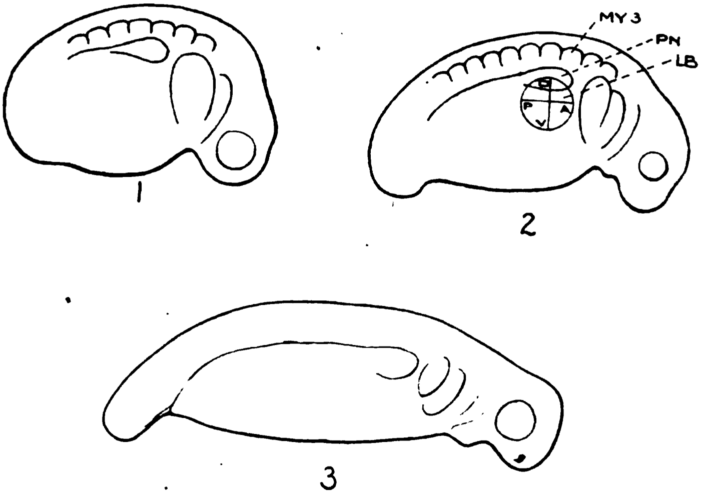
[7] A more complete analysis of the case is given by Detwiler ('18).

[8] Driesch, '99, '05, p. 679.

[9] In the absence of a set of 'normal plates' of Amblystoma, a series of stages have been designated arbitrarily and type specimens preserved. In course of time, if the necessary drawings can be prepared, it is hoped that they may be published.

plates may be observed through the skin. Though there is no distinct limb bud present, the region centering in the fourth segment just ventral to the pronephros contains the material that will give rise to the limb. A little later, in the standard operating stage when the tail bud is more marked (fig. 2, stage 29), sections show that the somatopleure ventral to the pronephros is thickened. Still we can scarcely speak of a 'limb bud' on the surface of the embryo, for the pronephric tubules are the chief cause of the swelling in that region, the somatopleural thickening merely serving to round it out on the ventral side. After this period the somatopleural proliferation increases, making the prominence on the side of the embryo more distinct (fig. 3, stage 33), but it is not until several days later that the extremity itself appears on the surface as a more sharp elevation in the



Figs. 1 to 3 Embryos of Amblystoma punctatum in the stages used for operation. × 10. *A*, *D*, *P*, *V*, mark the four cardinal points (anterior, dorsal, posterior, ventral) of the limb bud (*LB*); *MY3*, third myotome; *PN*, pronephros. Figure 1. Earliest stage (stage 25). Figure 2. Standard operating stage (stage 29). Figure 3. Oldest stage (stage 33).

region of the fourth somite ventral and somewhat caudal to the pronephros.[10]

The limb bud on its first appearance is a nodule about one and a half somites in diameter, and is almost radially symmetrical. It soon acquires greater convexity on its dorso-posterior border (fig. 4), and may be said to 'point' in that direction, though the surface is rounded all over. From this period on growth is rapid. The direction of 'pointing' becomes more marked and the tip of the bud frees itself from the body wall, the axis of the limb making an angle of 30°–35° with that of the body when viewed from above, and pointing dorsally at about the same angle to the horizontal. The bud elongates into cylindrical form, being attached to the body wall obliquely at its base. During this process the axis of the limb is bent more nearly parallel to the median plane (figs. 5 and 6). As the bending proceeds the distal part of the limb becomes flattened in a plane about 45° to vertical, the dorsal border being nearest the body. A little later the first trace of the digitations appears at the extreme tip of the limb, which becomes more squarish and then slightly concave, the depression representing the notch between the first two digits (fig. 7). The digits elongate rapidly, as does the whole limb, but the joints are at this time not very distinct. While the notch between the fingers is still shallow, the dorsal border of the limb becomes distinctly convex, and at the same time the hand is so twisted as to lie in a vertical instead of an oblique plane. The latter change is in reality partial pronation. The more lateral, which is morphologically the preaxial (radial) border, becomes ventral, the pollex lying on this side. The hand broadens out and the fore arm becomes somewhat flattened also. The elbow joint is now slightly flexed towards the ventral side (fig. 8). Up to this point the limb has no motility, the changes which have been described being due to growth and not to muscular action.

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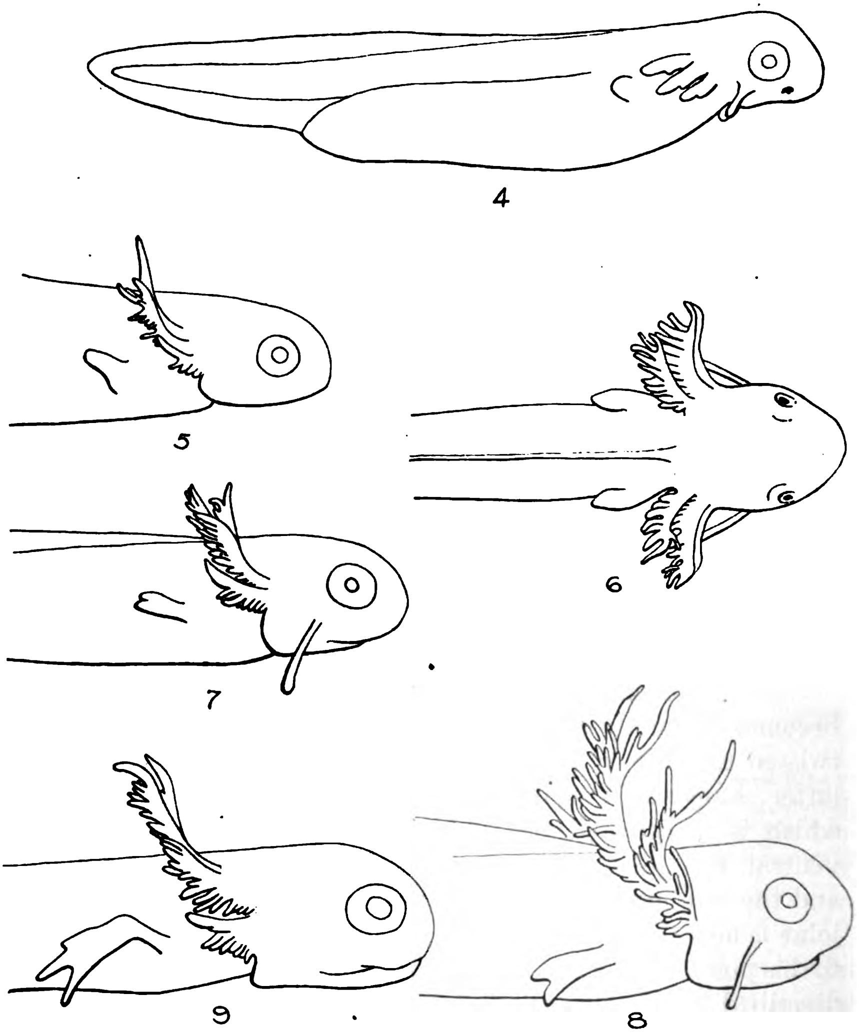
[10] The description of the normal development is given here merely for convenience, and only those phases which concern the experiments are considered. The subject is fully treated in papers by Götte ('79), Strasser ('79), Rabl ('01), with especial emphasis on external form and skeleton. Miss Byrnes ('98 a) has described the relation of the limb bud to the body wall and myotomes in the early stages.

The third and fourth digits appear successively on the ulnar (dorsal) border of the hand, first as nodules which slowly elongate, the fourth being considerably behind the third in its development (fig. 9). Viewed from above, the arm at this time is



Figs. 4 to 9 Normal embryos of A. punctatum, showing form and posture of the developing fore limb. Description in text. × 10.

seen to be bowed toward the body. Further changes in the form of the limb are concerned largely with the lengthening of the various segments, notably the digits, and the more distinct demarcation of the arm, fore arm, and manus.

Rotation takes place at the shoulder, the arm pointing more laterally and ventrally, so that the tip of the first digit rests on the bottom. Further rotation at this joint, coupled with flexion at the elbow, brings the manus much further forward beneath the gills, and the animal now rests upon two digits of each limb. The balancers, which serve to support the larva on its belly, are not lost till this stage is reached. The first muscular movements take place at the shoulder before this period, and later, movement at the elbow and wrist joints begins; the limb is then used in crawling, the positions just described being those of normal rest. These changes are completed just about the time the yolk is entirely gone and the larva begins to feed.

SIMPLE EXTIRPATION OF THE LIMB BUD

Mode of operation

The first experiment which will be considered consisted in the simple extirpation of the body wall of the limb region. To perform this operation the scissors are inserted through the outer layers of the embryo at the anterior (cephalic) border of the region to be removed and then the embryo is turned while a circular incision of the desired size is cut. The part of the body wall thus isolated may be readily lifted from the underlying tissue and removed in entirety. Care has to be taken at the upper border of the wound to disengage the limb rudiment from the pronephros, if the latter is to be saved intact. When the limb disc is lifted, both somatopleure and splanchnopleure come away with the overlying ectoderm, since the mesoderm has not split at that time. The operations were mostly done in the stage shown in figure 2, though the age of the embryos used varied from the stage shown in figure 1 to that in figure 3. No difference in the results, which may be ascribed to the difference in age within these limits, has as yet been noted. In the younger specimens the layers of the embryo are more readily separated, though in the older ones the tissues are of a firmer consistency and can be handled more satisfactorily.

After the disc is excised the wound at first gapes, but a few minutes later it contracts. Then in the course of the next twenty-four or forty-eight hours the ectoderm stretches itself over the wound bed and covers it entirely. There is much variation in the time required to complete this process, and in some cases the wound has been found still partly open four days after the operation. Sections show that the wound is first covered by ectoderm and that the mesoderm creeps in soon after between that layer and the yolk.

The first problem is to determine whether by an operation of this kind the development of the limb can be prevented, and, if so, how the size of the wound affects the outcome of the experiment. Two kinds of operations were done. In the first the limb disc was simply lifted and the wound left without further treatment. In the second the wound bed was afterwards carefully cleaned of all mesoderm cells. In some cases of each kind the pronephros was left intact and in others it was removed. Extirpation of this organ facilitates the cleaning of the wound, but since many cases of non-regeneration with intact pronephros occurred as well as some cases of regeneration without its presence, its influence, if any, upon the development of the limb must be unessential.

Relation of regeneration to size of wound and completeness of removal of mesoderm

The smallest wounds were three somites in diameter, encompassing in all but two cases the region ventral to myotomes 3, 4, and 5. In the two exceptional cases the wound, which was not cleaned, included the area below somites 4, 5, and 6; one of these regenerated and one died. The largest were 4½ somites in diameter, extending from the boundary between the second and third to the vertical line dividing the seventh myotome in half.

TABLE 1

Showing results of extirpation of circular areas of body wall of given diameter; wound not covered

DIAMETER OF EXTIRPATED AREA	WOUND NOT CLEANED					WOUND CLEANED				
	Cases regenerated	Cases not regenerated	Dead or discarded	Total	Per cent regenerated*	Cases regenerated	Cases not regenerated	Dead or discarded	Total	Per cent regenerated
Not recorded...	11	10	4	25 (21)	52.4	0	0	1	1 (0)	
3 somites......	19	1	4	24 (20)	95.0	13	12	9	34 (25)	52.0
3¼ somites.....	1	0	0	1 (1)		1	0	0	1 (1)	
3½ somites.....	111	23	81	215 (134)	82.8	3	18	11	32 (21)	14.3
4 somites......	14	2	2	18 (16)	87.5	2	3	13	18 (5)	
4½ somites.....	2	0	0	2 (2)						
Total........	158	36	91	285 (194)	81.4	19	33	34	86 (52)	36.5

* In calculating the percentages the number surviving and not the whole numbers of operations has been used. Percentages are given only in those classes where the number of cases is sufficiently large to be of significance.

The standard wound, which was made in the largest number of cases, was 3½ somites in diameter, including the area ventral to the third, fourth, fifth, and half of the sixth myotomes. The wounds of four segments in diameter took in usually the region extending from the third to the sixth somites, inclusive, though in one case the place of the wound was shifted one segment, and in ten other cases a half segment toward the head, thus including in the latter that portion of the body wall between the posterior half of the second somite and the anterior half of the sixth inclusive.

As shown in table 1, the result of the experiment depends in a certain measure both upon the size of the extirpated area and the completeness of the removal of tissue. Taking all of the experiments without reference to size, 81.4 per cent of the cases with ordinary wound regenerated limbs while only 36.5 with cleaned wounds did so. In both sets of experiments, increase in the size of the wound over three somites considerably reduces the proportion of regenerated limbs. High mortality of the large clean-wound class has considerably diminished the number of cases of this kind available. In these cases the healing is usually very slow. The yolk, being long exposed, begins to disintegrate after a time, and the embryo rarely recovers if this process sets in. It is possible that when a few mesoderm cells are left in, they cover the yolk and facilitate the overgrowth of the ectoderm.

Although the regenerative capacity is dependent to some extent on the size of the wound, there are experiments with wounds of each of the several sizes in which no regeneration took place. Within the limits of these operations it is therefore impossible to say that wounds beyond a certain size preclude regeneration altogether. This can only be said of those cases in which the wound is covered with indifferent skin, as described in another section (p. 432).

It is reasonably certain from a study of the normal development that the cells which ordinarily give rise to the limb bud take origin in the region below the fourth and the neighboring parts of the third and fifth somites. In this region, as the limb bud becomes more prominent, numerous mitoses are found, while the rest of the somatopleure is almost devoid of dividing cells. In those cases in which the region corresponding to the whole of these three somites, or even more, is removed the cells which give rise to the regenerated appendage are, therefore, probably such as in normal development do not participate in its development. Miss Byrnes ('98) has emphasized this in her paper on the hind limbs of Rana.

Relation of regeneration to age of embryo at time of operation

There seems to be no definite correlation between the age of the embryo at the time of operation and the occurrence of regeneration. The tabulation here given (Table 2 a), which includes both covered and uncovered wounds shows that when cases are recorded in significant numbers, both positive and negative results are obtained after operation in both older and younger stages.

The number of cases operated in the extreme stages is small and no statistical value can be placed upon the figures there.

TABLE 2A

Showing frequency of regeneration after operations in the several stages

STAGE	WOUND NOT CLEANED			WOUND CLEANED			ALL EXPERIMENTS COMBINED		
	Cases regenerated	Cases not regenerated	Per cent regenerated	Cases regenerated	Cases not regenerated	Per cent regenerated	Dead or discarded	Total cases	Per cent regenerated
25	4	0		0	1		2	7 (5)	
26	3	0		2	3		0	8 (8)	
27	8	2	80.0	3	11	21.4	4	28 (24)	45.8
28	20	0	100.0	2	11	15.4	20	53 (33)	66.7
29	65	12	84.4	10	25	28.6	41	153 (112)	66.1
30	26	5	83.9	5	9	35.7	28	73 (45)	68.9
31	12	3	80.0	2	3		28	48 (20)	70.0
32	4	4		2	0		4	14 (10)	60.0
33	14	10	58.3	1	0		4	29 (25)	60.0
Miscellaneous*	4	5		0	2		6	17 (11)	
Total.........	160	41		27	65		137	430 (293)	

* Stage not recorded or else outside above limits.

TABLE 2B

Showing frequency of regeneration after operation in the younger and older groups of stages, respectively

STAGE	WOUND NOT CLEANED			WOUND CLEANED			ALL EXPERIMENTS COMBINED		
	Cases regenerated	Cases not regenerated	Per cent regenerated	Cases regenerated	Cases not regenerated	Per cent regenerated	Cases regenerated	Cases not regenerated	Per cent regenerated
Stage 29 or under...	100	14	87.7	17	51	25.0	117	65	54.3
Over stage 29.......	56	22	71.8	10	12	45.5	66	34	66.0

One point stands out, however, which may possibly be of significance, though no altogether satisfactory explanation is apparent. In the group with wounds not cleaned a higher percentage of regenerating cases is found among those operated in the younger stages, while in the group with cleaned wounds the higher percentage is at the opposite end of the series. This is brought out more clearly when the experiments are divided into but two classes, comprising respectively the cases operated when older than the standard operating stage and those in or below it (Table 2 b). The lower percentage of regeneration in

TABLE 3

Showing time factor for regeneration in the different classes of experiments

CHARACTER OF OPERATION	NUMBER OF CASES	AVERAGE TIME AFTER OPERATION REGENERATION FIRST OBSERVED	AVERAGE TIME AFTER OPERATION LAST PREVIOUS OBSERVATION	NUMBER OF CASES REGENERATION FIRST OBSERVED AT 10 DAYS OR LATER	NUMBER OF CASES REGENERATION FIRST OBSERVED AT 15 DAYS OR LATER	OLDEST STAGE NEGATIVE OBSERVATION FOLLOWED BY REGENERATION
		days	*days*			*days*
Wound not covered:						
Not cleaned......	158	6.8	2.8	21	4	10
Cleaned..........	19	9.2	5.4	7	2	15
Wound covered:						
Not cleaned......	2	13.5*	2.5*	1	1	4
Cleaned..........	8	9.4	6.0	3	1	11

* The figures in this class have no significance since in one of the two cases no observation was recorded between the fourth and the twenty-third day.

the older embryos in the not-cleaned series may be due to the fact that the limb bud can be lifted out more cleanly than in the younger stages, so that after-cleaning is less needed. The higher percentage of regeneration after operations in the older stages in the cases with cleaned wounds remains, however, anomalous.

Delay in development after extirpation

Removal of the limb rudiment, if it does not arrest development entirely, naturally retards it to a considerable degree, the delay being on the average greater in the case of the cleaned wounds than in the others (Table 3). In a few cases outward signs of regeneration were noted as early as four days after operation, though usually not until later. Since it was not practicable to observe the embryos each day, it cannot be stated exactly when regeneration did begin in each case. One hundred and fifty-eight cases with ordinary wounds show that the regenerating limb was first noted on the average 6.8 days after the operation, the last previous observation having been made on the average at 2.8 days. In the case of the cleaned wounds, the first observation of regeneration averaged 9.2 days after operation, the last previous observation having been at 5.4

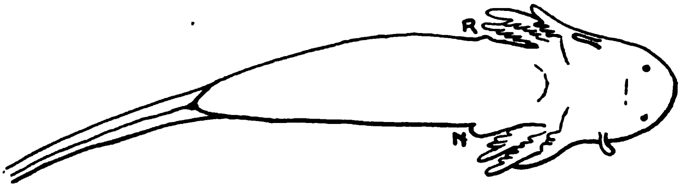
days. This is an average of nineteen cases. Daily observation would undoubtedly have revealed a greater difference between the two classes of cases in time of appearanec of the regenerating limb bud. In a total of 177 cases there are but twenty-eight in which regeneration was first noted as late as ten days after the operation, and but six when the period was fifteen days or over. In only four of the twenty-eight cases was the last previous observation made as late as ten days after the operation, and only once was a negative observation, with subsequent regeneration, recorded as late as fifteen days. It is safe to conclude, then, that when regeneration has not become visible from the outside two weeks after the operation it will not occur at all. Nearly all of the cases have been kept alive at least three weeks, many for four, and some as long as twelve (fig. 14), and no instances of regeneration in the later periods of development have been observed.

The experiments described in this section show that it is not possible to prevent the regeneration of the fore limb with certainty when the wound is left uncovered, even if the circular area extirpated has a diameter of 4 somites. It may be said in general, however, that the larger the area removed and the more carefully the wound is cleaned of mesoderm the less likely will regeneration occur. This can only mean that the mesoderm cells of the region surrounding the limb bud have, in a gradually diminishing degree as the distance from the limb increases, the potency to form a limb. Their prospective potency is, therefore, greater than their prospective significance. Regeneration, when it takes place, is usually perfect, though subject to delay in a varying degree.

The process of regeneration

The actual process of regeneration has not been followed in detail, though some observations may be recorded here.

The earliest case (R. E. 127–) which need be considered was preserved six days after extirpation of the limb rudiment. The regenerating limb appears on the surface (fig. 10) as a small nodule ventral and posterior to the pronephros. In sections it is seen to consist of closely packed cells which have approximately the same yolk content as those of the normal limb on the opposite side and which show numerous mitoses. This indicates that after the defect is covered up by inwandering of peripheral cells (p. 422), the process of regeneration, like the original development of the limb, is dependent upon multiplication of cells in situ rather than upon the continued crowding together of cells from the surrounding regions. The ectodermal covering of the new limb bud, as well as that of the original one, is considerably thicker than the ectoderm of the neighboring region of the trunk.



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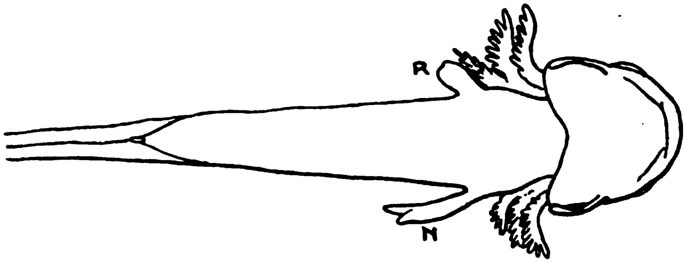

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Figs. 10 and 11 Embryos with regenerating limb buds, ventral view. × 10. *R*, regenerating limb; *N*, normal limb. Figure 10. Experiment R. E. 127–, six days after operation. Figure 11. Experiment R. E. 129–, fifteen days after operation.

A case (R. E. 115–) preserved at nine days shows similar conditions, though further advanced. Nerve fibers from two spinal nerves may be traced to the base of the regenerating limb.

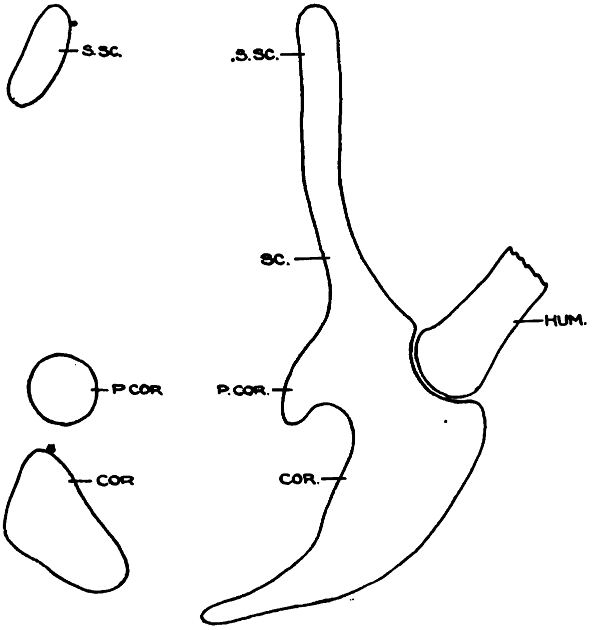
A larva killed fifteen days after operation (R. E. 129–, fig. 11) shows traces of digits and the segregation of some of the skeletal elements, though no marked differentiation of tissues. Mitoses are still very numerous and the blood vessels within the limb are unusually large. It is odd that there is as yet no trace of the shoulder girdle, which is already well developed on the normal side. In normal limbs of the size of the regenerating one in this specimen, the girdle can be readily made out.

The shoulder girdle in the absence of the limb

In cases where the free limb does not regenerate there is a partial development of the shoulder girdle. This is shown in every case that has been examined, confirming Braus's observations on the Anura.

Some of these cases have been studied in serial sections and others in total preparations of the shoulder region (figs. 12 and 13). The cartilaginous elements found are *a*) the coracoid (*COR*), which has the same general shape and position as the normal, but is not so large; *b*) a small nodule, dorsal to the coracoid at the point where the humerus would normally articulate, representing the procoracoid (*P. COR*), and *c*) a rod-shaped element, external to the pronephros, which is entirely separate from the rest of the girdle and which may be identified with the suprascapula (*S. SC*). The scapula is absent. The ventral trunk musculature is approximately normal, though thinner in the girdle region, and usually it is partly interrupted. A distinct band of muscle running from the ventral end of the suprascapula dorsally and anteriorly just under the epidermis is to be identified with the *m.* trapezius, though in the normal girdle this muscle is attached further ventrally. The conditions found change with the age of the specimen.

In a case preserved eighteen days after extirpation of the limb bud (E. E. 61) there are only two small nodules of cartilage, or rather precartilage, present. One is at or slightly ventral to the level of the normal shoulder joint and represents the coracoid, and the other is between the muscle plates and skin at the level of the notochord, opposite the dorsal extremity of the normal suprascapula, which it undoubtedly represents in rudimentary form. The pronephros had been removed in this case so that the relation to this organ could not be exactly determined.



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Figs. 12 and 13 Outline of shoulder girdle cartilages of an embryo (Experiment E. E. 59), from which the fore limb bud had been removed on the right side and had not regenerated. Specimen preserved twenty-seven days after operation. From a total preparation of the shoulder region. × 60. The cartilages are represented in lateral view projected upon the sagittal plane. *COR.*, coracoid; *HUM.*, humerus; *P.COR.*, procoracoid; *SC.*, scapula; *S.SC.*, suprascapula. Figure 12. Operated right side. Figure 13. Normal left side.

Another specimen (E. E. 26), preserved twenty-four days after operation, shows both the dorsal and ventral elements much better developed. The suprascapula is a long rod of cartilage extending from near the level of the *n. lateralis* ventrally almost to the level of the glenoid cavity of the unoperated side. The coracoid is more massive than in the previous case. The procoracoid is also present, being represented by a small nodule of cartilage anterior to the ventral end of the suprascapula. The three cartilages are distinct from one another, but are connected by fascia or ligaments.

A case (E. E. 52) twenty-eight days old has a still heavier girdle, especially the ventral portion, which is almost as large as the normal. The procoracoid is fused with the coracoid, and these two ventral elements are not separated very widely from the suprascapula, although the latter cartilage extends hardly so far ventrally as in E. E. 26.

An individual which lived thirty-three days from the time of operation (E. E. 71) shows the suprascapula very well developed, reaching ventrally nearly to the level of the shoulder joint. However, the ventral part of the girdle is relatively poorly developed in this case, the coracoid and procoracoid being small and still separated from one another.

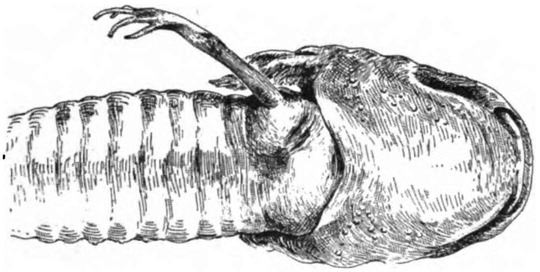


Fig. 14 Amblystoma larva (Experiment Tr. E. 53) from which the left limb bud had been removed and not regenerated; preserved eighty-seven days after operation; ventral view. × 5.

In a case preserved thirty-nine days after operation (Tr. E. 62–) the dorsal portion of the girdle is well developed and at its ventral end enters into cartilaginous union with the posterior end of the procoracoid. The latter approaches close to the coracoid cartilage and is united to it by a strong ligamentous attachment, though there is no continuity of cartilaginous substance. This is in contrast to E. E. 52, where the coracoid and procoracoid are fused and the suprascapula separate.

The oldest case examined (Tr. Ext. 61–) was killed eighty-five days after operation (fig. 14, taken from another specimen of like age). In this the shoulder elements are united into a single cartilage, extending from the level of the dorsal surface of the medullary cord to the ventral mid line. On the unoperated side the cartilage is considerably stouter.

Taken together with the results of Detwiler's studies on the development of the shoulder girdle after extirpation of various portions of the limb region, these cases indicate that the operation used in extirpating the limb rudiment leaves in the embryo the rudiments of the suprascapula, the coracoid and the procoracoid. These differentiate into small cartilages, which by a process of hyperplasia gradually extend across the gap intervening between them until they become united into a girdle which is topographically complete.

THE EFFECT OF COVERING THE WOUND WITH SKIN FROM ANOTHER REGION OF THE BODY

In order to block the stimulus to regeneration, presumed to arise from the presence of a defect, the wound made in extirpating the limb rudiment was in a number of cases covered with indifferent skin from the body of another embryo. The covering was taken either from the flank, tailward from the anterior limb region, or from the belly, and consisted of ectoderm with at least a few mesoderm cells attached. In transplanting embryonic skin it is necessary to work quickly. The major operation must be done first and the embryo placed in position ready to receive the graft. Then the skin is excised from another specimen, and transferred directly to the wound in the first embryo, where it must be pressed into place immediately by a silver or glass bar. If this is not done quickly the skin rolls itself into a ball and cannot be used.

Such experiments were made mostly with cleaned wounds of various sizes. The results are given in Table 4, which should be compared line for line with Table 1. From the comparison of the two tables it is seen that covering the wound has a strong tendency to inhibit regeneration. For cleaned wounds of 3 somites in diameter the percentage of regenerating cases is reduced from 52 to 33. When the wound is $3\frac{1}{2}$ somites or more

TABLE 4

Showing results of extirpation of circular areas of body wall of given diameter; wound covered with grafted skin

DIAMETER OF EXTIRPATED AREA	WOUND NOT CLEANED					WOUND CLEANED				
	Cases regenerated	Cases not regenerated	Dead or discarded	Total	Per cent regenerated	Cases regenerated	Cases not regenerated	Dead or discarded	Total	Per cent regenerated
Not recorded	2	5	3*	10						
3 somites						7	14	3	24 (21)	33.3
3¼ somites						1	0	0	1 (1)	
3½ somites						0	15	4	19 (15)	00.0
4 somites						0	3	2	5 (3)	
Total	2	5	3	10		8	32	9	49 (40)	20.0

* These three cases were classed as negative in the tabulation previously published (Harrison, '15). They are excluded here because they were not kept under observation for a sufficient length of time.

in diameter, regeneration is altogether blocked, eighteen cases all having given a negative result. To these might be added the five cases given in Table 8, which differ from the present experiments only as to the region from which the covering ectoderm is taken (p. 448).

When the skin is grafted to the wound it soon sticks to the underlying tissue just as a transplanted limb bud does, and often in the course of several hours the wound becomes completely healed. The wandering of the ectoderm over the denuded area, which takes place in the uncovered wounds, is blocked, and probably also the movement of the mesoderm cells. Prevention of the surrounding cells from reaching the proper position by the substitution of other tissues which do not have the potency to produce a limb thus effectually prevents the regeneration of the appendage. The cells immediately surrounding the limb-producing area are evidently unable to form a limb unless they can reach the proper position. On the other hand, indifferent cells in this position cannot produce a limb. It is possible that when no mesoderm is grafted with the skin, the cells of this layer wandering in from the host may in some cases give rise to a limb. Some of the individual discrepancies in this series of experiments may be due to unconscious varying of this factor.

In experiments in which another limb bud is transplanted into the place of an extirpated normal bud, the grafted tissue must act like a piece of body wall from an indifferent origin in so far as its effect upon the movement of the cells of the host is concerned. It must prevent these cells from wandering into the proper position to form a limb, and hence when transplantations of the limb bud are undertaken with proper precautions as to size of wound and thorough cleaning of the mesoderm, it is safe to assume that the limb that does develop arises from the transplanted material and not from the tissues of the host. The exact determination of the size and character of the wound necessary to prevent regeneration is therefore important for the proper interpretation of any experiments in which the normal limb bud is replaced by a grafted one.

EFFECT OF REMOVAL OF PORTIONS OF THE LIMB BUD

It was scarcely to be expected that an organ having such marked regenerative capacity as the limb rudiment would show any distinct localized effect of the removal of definite portions. A number of experiments have nevertheless been made to test the prospective potency of its parts. The procedure was as follows: The limb area was first bisected by a vertical or a horizontal incision and half of the disc—anterior or posterior, dorsal or ventral—was removed. Some of the wounds were left without further treatment; in others the mesoderm was carefully cleaned off and the wound left to heal; and in still others the denuded area was covered with ectoderm from the flank of another embryo just as in the experiments with whole limb buds. In a smaller number of cases, a small circular area, 1 or 1½ somites in diameter, was removed from the center of the limb rudiment. The first experiments, which were referred to in the preliminary paper (Harrison, '15), were few in number and were made without special cleaning of the wound. They resulted in the development of normal or only slightly defective

TABLE 5

Summary of results of removal of portions of the limb bud. Wounds cleaned

PART REMOVED	RESULTING LIMB						
	Normal	Redupli-cated	Defective	Abortive	Dead	Total	Per cent normal
Anterior	3	6	0	1	4	14 (10)	30.0
Posterior	2	1	3	4	3	13 (10)	20.0
Dorsal	1	0	1	10	2	14 (12)	8.3
Ventral	8	0	0	2	4	14 (10)	80.0
Central	4	1	1	0	1	7 (6)	66.7
Total	18	8	5	17	14	62 (48)	37.5

TABLE 6

Summary of results of removal of portions of the limb bud. Wounds not cleaned

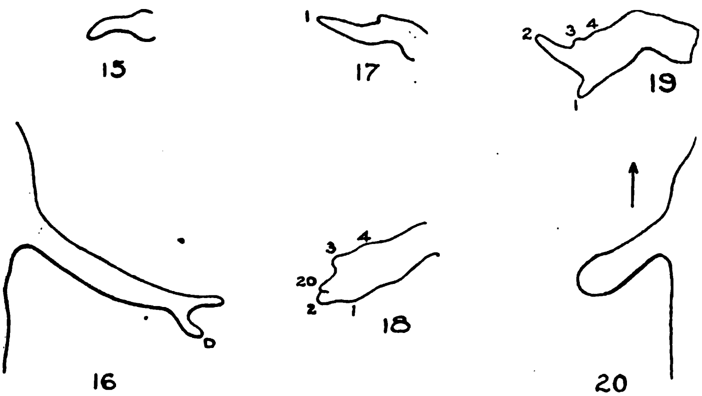
PART REMOVED	RESULTING LIMB						
	Normal	Redupli-cated	Defective	Abortive	Dead	Total	Per cent normal
Anterior	12	0	1	0	4	17 (13)	92.3
Posterior	6	0	4	1	4	15 (11)	54.5
Dorsal	5	0	1	4	1	11 (10)	50.0
Ventral	7	0	1	0	2	10 (8)	87.5
Central	1	0	1	0	0	2 (2)	50.0
Total	31	0	8	5	11	55 (44)	70.5

limbs, but cannot be regarded as a fair sample of what the operation may effect.

During the season of 1917 the experiments have been greatly augmented in number, and with results which seem rather different. There has been a large proportion of cases with defective or completely inhibited development of the operated limbs, although no specific correlation between the part removed and the character of the defect has been observed. As in the experiments with whole limb buds, a much higher percentage of perfect limbs has resulted when the wound is not cleaned. The results of the experiments in the two classes of cases are given in Tables 5 and 6.

Those with cleaned wounds (Table 5), including all which were covered with grafted skin, will be considered first. In this group it is obvious that the proportion of normal limbs varies very greatly among the different operations. Thus when the ventral half of the limb bud was removed, eight out of ten resulted in perfect limbs; but when the dorsal half was taken out, only one out of twelve gave rise to a normal appendage. The anterior and posterior halves occupy an intermediate position between the two extremes. These discrepancies are largely, if not entirely, due to the difficulty of exactly halving the material that is to form the limb. In bisecting it vertically the myotomes were used as a guide, and in most cases the incision was made below the middle of the fourth somite, leaving one and a half somites in front of the incision and two behind it. This seems to divide the material more nearly in half than when the incision is made a quarter somite further back. In the case of the horizontal incision it is more difficult to divide the rudiment accurately, because there is no sharply defined landmark. The attempt was made to cut a little below the pronephric swelling. The results show that more of the limb material lies above the cut than below. In other words, if the circular area, centering just below the pronephros and extending from the boundary between the second and third somites to half way through the sixth, is bisected vertically and horizontally, more limb-forming material lies dorsal to the line than ventral, and more anterior than posterior. The lines shown in figure 2 designate more nearly the exact quartering of the material.

The fact that normal appendages resulted in some cases after each kind of operation shows that there is no localization of prospective potencies in the operating stage. Examination of the character of the defects that do arise confirms this conclusion. The large proportion of the latter are defects of the whole limb, which remains a nodule or becomes entirely resorbed. They must be ascribed to the general effect of the operation and not to the removal of any specific material. Seventeen cases out of a total of forty-eight operations (not including cases that died) with cleaned wounds resulted in this way. Of the partial defects, listed under the caption 'defective,' all five affected the hand, especially the digits. The most marked case was one in which the dorsal half of the limb bud was removed (Rem. E. 27). This was a club-shaped appendage less than half length and without hand (fig. 15). The next most marked defect followed removal of the posterior half of the bud (H. R. E. 12–); here the forearm was slender and but one whole digit was developed, which, however, had a reduplicating branch (fig. 16). In another case of removal of the posterior half (Rem. E. 13) only a single long digit and the stump of a second were developed, the hand being quite defective (fig. 17). Amputation of this incomplete limb was followed, however, by the regeneration of a normally formed one. The two remaining cases showed defects

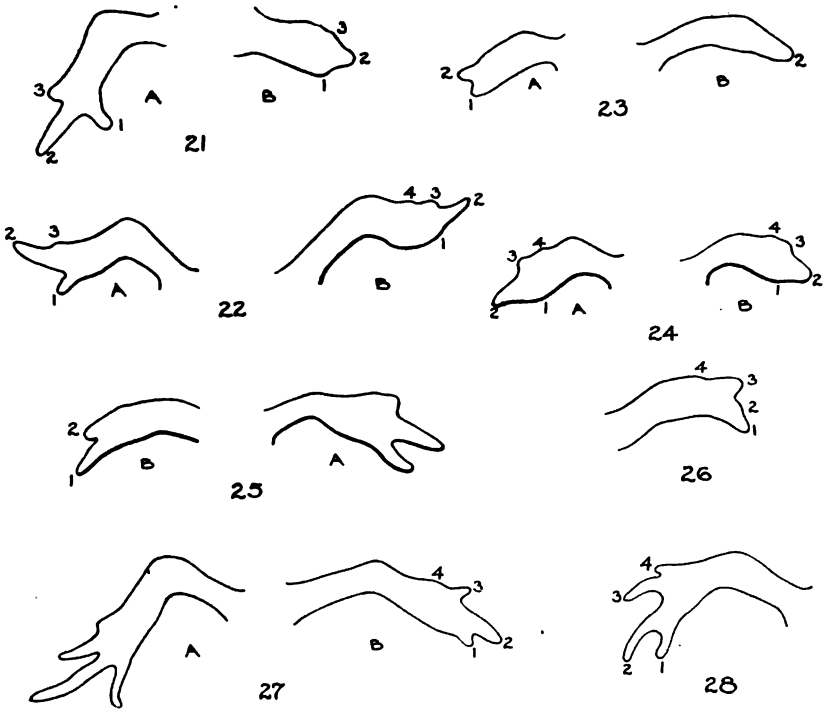


Figs. 15 to 20 Outline views of defective limbs which developed after removal of portions of the limb bud. × 17. *1, 2, 3, 4,* ordinal number of the digits; *d.*, reduplicating digit. Figure 15. Experiment Rem. E. 27 (dorsal half removed); lateral view right side, considerably foreshortened; twenty-six days after operation. Figure 16. Experiment H. R. E. 12– (posterior half removed); ventral view; twenty-six days after operation; the arrow points headward. Figure 17. Experiment Rem. E. 13 (posterior half removed); limb amputated and preserved twenty-seven days after operation; ulnar half of hand is very defective, but the limb which regenerated after amputation was normal. Figure 18. Experiment Rem. E. 29 (posterior half removed); lateral view right side, arm much foreshortened; first digit (*1*) abortive and syndactylous with second, which has a reduplicating branch (*2D*); thirty-one days after operation. Figure 19. Experiment Rem. E. 21 (central portion of limb bud removed); limb amputated and preserved twenty-six days after operation; right limb lateral view. Figure 20. Experiment H. E. 21 – (anterior half removed); right side ventral view, eighteen days after operation; the arrow points headward.

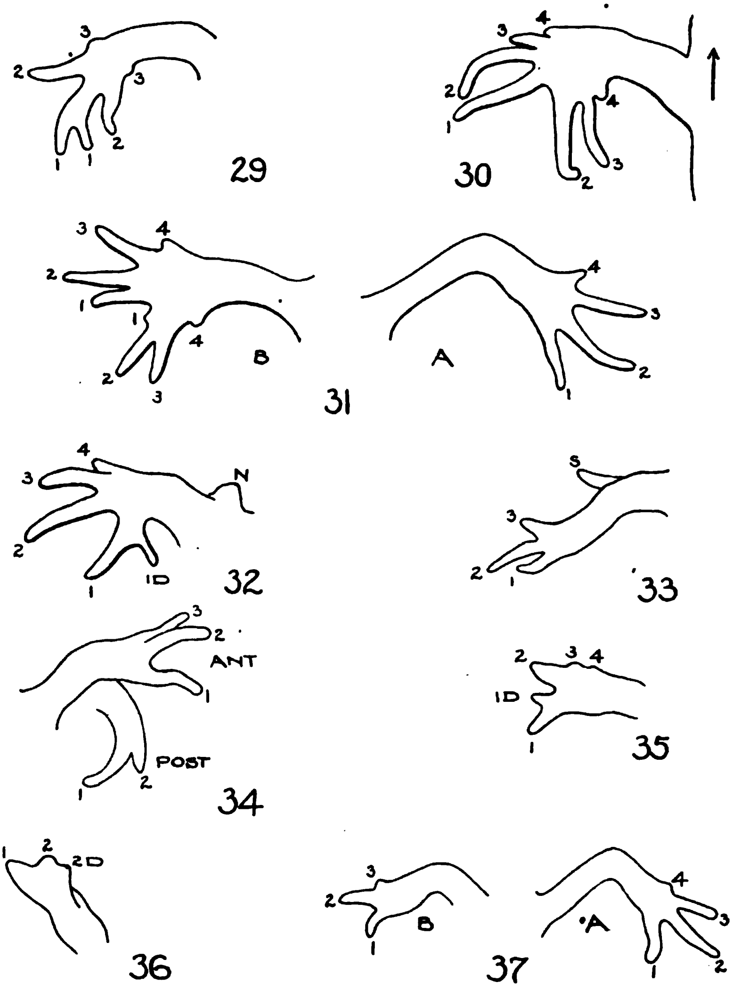
in the first digit. In one (Rem. E. 29), where the posterior half of the bud had been extirpated, the first digit is a mere stump and is syndactylous with the second, which, however, has a small reduplicating branch on the ulnar side (fig. 18); in the other (Rem. E. 21), from which the central portion had been excised, the first digit is short (fig. 19).

The cases with ordinary (not cleaned) wounds (Table 6), like the foregoing, show the highest proportion of defects after removal of the dorsal half of the limb bud. However, the lowest proportion of defectives occurred after removal of the anterior half, a result for which there is no apparent explanation. Removal of the dorsal portion gives a relatively large number of cases of complete suppression of the limb. Of the partial defects, the most marked case (H. E. 21–) has the hand totally lacking, the arm ending as a rounded stump (fig. 20); here the anterior portion had been removed. Another case (H. E. 13–, posterior half removed) appears similar though less clear. In four of the remaining, two of which followed extirpation of the posterior (Rem. E. 2 and 7), one of the ventral (Rem. E. 1) and one of the central (Rem. E. 9) portion of the bud, the defect involves only the first digit, which is either absent or short (figs. 21 B–24 B), while the other two cases (H. E. 18–, removal of dorsal half, and H. R. E. 33–, removal of posterior half) have the second digit short (figs. 25 B and 26). One of the cases (Rem. E. 9) shows the same defect on both the operated and the unoperated sides (figs. 24 A and B). In a few of the cases, webbing of the first two fingers occurred (figs. 27 B and 28). This has at times been found in other experiments and even in unoperated limbs, and since the deviation is slight these cases have been classed among the normal.

Turning to the reduplications, we find them concentrated among the cases in which the anterior half of the limb bud had been removed, six out of the eight falling within this group. They include a variety of forms, such as two separate and nearly complete limbs (fig. 34), a single normal limb with a spur attached to the upper arm (fig. 33), and a single limb with merely a branched or double digit (fig. 35). Three of them (Rem. E.



Figs. 21 to 28 Effect of removal of portions of the limb bud (continued). In the double figures *A* represents the limb of the unoperated and *B* that of the operated side. In all cases lateral view. × 17. Figure 21. Experiment Rem. E. 2 (removal of posterior half); upper arm unoperated limb (*A*) much foreshortened; operated limb (*B*) generally less advanced in development, and with abortive first digit (*1*); seventeen days after operation. Figure 22. Experiment Rem. E. 7 (removal of posterior half); digits of normal limb (*A*) foreshortened in part; first digit of operated limb abortive; twenty-six days after operation. Figure 23. Experiment Rem. E. 1 (removal of ventral half); unoperated (*A*) limb not so long as operated (*B*); which has only one long digit; twenty-nine days after operation. Figure 24. Experiment Rem. E. 9 (central portion removed); both limbs foreshortened; unoperated limb further advanced, but both show defect of first digit; twenty-six days after operation. Figure 25. Experiment H. E. 18 – (dorsal half removed); limb of operated side has short second digit (*2*); eighteen days after operation. Figure 26. Experiment H. R. E. 33 – (removal of posterior half); second digit (*2*) is abortive and fused with the first (*1*); third digit well developed; twenty-one days after operation. Figure 27. Experiment Rem. E. 3 (anterior half removed); operated limb somewhat less developed than normal; syndactyly of digits 1 and 2; twenty-seven days after operation. Figure 28. Experiment H. E. 28 – (removal of posterior half); well-developed limb with syndactylous first and second digits; twenty-eight days after operation.



Figs. 29 to 37 Effect of removal of portions of the limb bud (continued). Lateral view of limbs in all figures except figure 30. × 17. Figure 29. Experiment Rem. E. 48 (removal of anterior half); arm foreshortened, but hand not; radial reduplication of hand; twenty-seven days after operation. Figure 30. Experiment H. E. 6 – (anterior half removed); ventral view; radial reduplication of hand more complete than in last case; thirty-nine days after operation; the arrow points headward. Figure 31. Experiment Rem. E. 52 (removal of anterior half); *A*, normal tetradactylous limb; *B*, operated limb, with double hand, considerably foreshortened; twenty-seven days after operation. Figure 32. Experiment Rem. E. 28 (anterior half removed); arm considerably foreshortened, probably reduplicated internally; first digit double (*1D*); *N*, nodule at

48, Rem. E. 52, and H. E. 6–) show more or less complete symmetrical reduplication of the hand (figs. 29, 30, and 31). Another (Rem. E. 28) has only the first digit doubled externally (fig. 32), though the unusual thickness of the limb indicates partial internal reduplication. Near the shoulder there is a distinct nodule. The case of the single limb with spur (Rem. E. 17) is interesting in that the spur developed out of a bud which grew posterior to the wound scar and which at first seemed to be the main limb. Anterior to the wound a second bud appeared, first noticed eight days after the operation. On the twelfth day it was still but a nodule, and not till the eighteenth day did it look like a regenerating bud. It finally developed into a normal limb of the proper laterality, the posterior bud remaining attached to it as a spur (fig. 33). The case with two independent limbs (H. R. E. 10–) is fundamentally similar to the foregoing. The main limb bud developed posterior to the wound. Later there appeared anterior to this a second bud. In this case, however, the two buds remained permanently separate. The anterior one gained over the posterior and became a normal limb of proper laterality with good function (fig. 34). The posterior one remained somewhat defective (second digit short and ulnar digits undeveloped), and when last examined alive, thirty-eight days after operation, it showed no movement. The remarkable feature of this case is that both limbs are right-handed, as was probably true of the spur case also, though the spur is too de-

shoulder; thirty-one days after operation. Figure 33. Experiment Rem. E. 17 (anterior half removed); hand foreshortened dorso-ventrally; *S*, spur representing a duplicate limb; forty-three days after operation. Figure 34. Experiment H. R. E. 10 – (removal of anterior half); two left limbs; *ANT.*, anterior member, developed secondarily from anterior border of wound; *POST.*, posterior member developed from the remaining half of the limb bud; forty days after operation. Figure 35. Experiment Rem. E. 18 (posterior half removed); radial digit reduplicated (*D*); limb amputated and preserved twenty-seven days after operation, followed by regeneration of normal limb (fig. 37 *b*). Figure 36. Experiment Rem. E. 16 (central portion removed); reduplicated second digit (*2D*); twenty days after operation. Figure 37. Experiment Rem. E. 18 (posterior half removed); *A*, normal left limb, *B*, normal right limb, regenerated after amputation of abnormal limb shown in figure 35; thirty-three days after amputation.

fective to reveal its laterality. This case differs from the three described above in which the hand is symmetrically reduplicated, one member being a right and one a left, following the rule of mirroring. It is probably of a fundamentally different nature from the others in that the posterior member obviously arose from the remaining half of the limb bud after operation, while the anterior one regenerated from the anterior border of the wound, the two remaining far enough apart not to influence one another. In the case of the true reduplications, the two members presumably arise from a single center which later doubles symmetrically. The other two cases of reduplication are not important. One (Rem. E. 18) involved the first digit only (fig. 35), and the other (Rem. E. 16) the second digit (fig. 36).

In three cases the abnormal appendages which developed were amputated between the shoulder and elbow. One of them (Rem. E. 13, with a very defective hand with only one digit (fig. 17)) and another (R. E. 18 with a reduplicated and a defective digit (fig. 35)) regenerated normal limbs (fig. 37). The other failed to regenerate. These experiments show that such anomalies can hardly be due to the removal of specific organ-forming tissues from the rudiment.

The anomalies are summarized in table 7. From this tabulation it is seen *a*) that defectiveness of the first digit may occur after removal of the posterior or the ventral halves or the central portion of the limb rudiment; *b*) that defectiveness of the whole hand may arise after removal of the anterior, posterior, or dorsal halves; and *c*) that abortive limbs may occur after removal of any of the four halves. As for the reduplications, those of major degree are confined to operations on the anterior half of the limb bud. Minor reduplications, affecting the digits only, occurred in one case after each of three different operations.

It would require a number of experiments many times that included in the above table to give statistical value to the numbers in the several categories, and it is not likely that these can be done in the near future. Possibly the repetition of the experiments on a large scale might show, for instance, a relatively high proportion of defects in digits after removal of the posterior

TABLE 7

Showing distribution of anomalies among the several experiments in removing portions of the limb bud

CHARACTER OF ANOMALY	PART REMOVED														
	Anterior			Posterior			Dorsal			Ventral			Central		
	Cleaned	Not cleaned	Total	Cleaned	Not cleaned	Total	Cleaned	Not cleaned	Total	Cleaned	Not cleaned	Total	Cleaned	Not cleaned	Total
Syndactyly of first two digits*		2	2		2	2									
First digit defective or absent				1	2	3					1	1	1	1	2
Second digit defective					1	1		1	1						
Hand defective				1		1									
Forearm and hand defective				1	1	1									
High degree of defectiveness—hand absent		1	1				1		1						
Reduplicated digit	1		1	1		1							1		1
Reduplicated hand mirrored	3		3												
Limb with reduplicating spur	1		1												
Two distinct limbs not mirrored	1		1												
Whole limb abortive or resorbed	1		1	4	1	5	10	4	14	2		2			
Total anomalies	7	1	8*	8	5	12*	11	5	16	2	1	3	2	1	3
Normal excl. syndactyly	3	10	13	2	4	7	1	5	6	8	7	15	4	1	5
Per cent normal*	30.0	92.3	65.2	20.0	54.5	42.9	8.3	50.0	27.3	80.0	87.5	83.3	66.7	50.0	62.5

* As in tables 5 and 6 syndactyly is classified with the normal.

half, though the small figures given in the table cannot be deemed significant in this direction. Moreover, this defect, like that of syndactyly, has been found in cases which had not been operated upon at all, and is probably to be regarded as due to slight general disturbances of growth.

On the other hand, the removal of the anterior portion seems to have a definite tendency to bring about reduplication, which is probably due to a more or less complete separation of the remaining portion of the limb rudiment from a regenerative center in front of the wound scar.

Notwithstanding these anomalies, the experiments speak as a whole for the equipotentiality of the four quadrants of the limb bud, at least in a qualitative sense. Quantitatively, the lines which divide the limb-forming tissue equally are anterior, and dorsal to the vertical and horizontal diameters, respectively, of the limb bud as defined by the experiments (fig. 2).

These statements are valid for the free extremity only, and must be held in abeyance with respect to the shoulder girdle, where it is known that localization has taken place in the embryo at the time of operation (p. 429).

While the prospective potency of the limb-forming cells is the same as regards the topographic divisions of the limb, the experiments give no evidence regarding histogenetic potencies. Whether certain cells at the operating stage are already differentiated into cartilage, bone, connective tissue, or muscle-forming elements cannot be determined either by direct observation or by any of the experiments yet devised.

What the prospective significance of the cells of each of the four quadrants of the limb bud is, i.e., what part of the free extremity is formed in normal development out of each, has not yet been determined, though the study of normal embryos points to the view that the distal part of the limb is developed more particularly from cells lying in the posterior half, the ulnar half arising from the dorsal and the radial from the ventral quadrant. It is difficult to devise experiments to test this hypothesis. Grafting of tissue colored by vital stains, such as neutral red and Nile blue sulphate, is not feasible because the stain is all deposited in the ectoderm. A few experiments have been made by grafting markers in the form of pieces of notochord, which could readily be recognized, in the mesoderm of the different quadrants of the limb bud, in the hope that they might be located after the limb was developed. As yet these have resulted negatively.

ATTEMPTED SPLITTING OF THE LIMB BUD

Some years ago, in experimenting with larvae of the anuran, Pelobates, Tornier ('05) found that by making a deep incision through the hind-limb rudiment and the base of the tail he could produce double appendages.

A few experiments have been made for the same purpose in connection with the present study. The material has necessitated, however, a rather different mode of operation, and the results have proved to be different.

The limb bud was deeply incised through the middle, either dorso-ventrally or antero-posteriorly, and a narrow strip of tissue including both ectoderm and mesoderm was removed. Thirteen experiments were made. Six were lost by accident eleven days after the operation, but all of them had at that time normal limbs on the operated side. The other cases were kept for sixteen or eighteen days and again all had normal limbs, though in four cases development was somewhat retarded. Nine of the embryos were in the oldest stage used (fig. 3) at the time of operation. The wound usually left a distinct scar or groove running across the limb bud, which, however, was obliterated after several days. In no case did the operation result in reduplications. The difference between these results and those of Tornier may be ascribed to the fact that in the case of the latter the operation was more radical and done upon older embryos, so that the chance of the divided limb rudiment healing together completely was much less.

EFFECT OF SUPERIMPOSING LIMB BUDS

In experiments upon early embryonic stages the most usual test of equipotentiality of the parts has been the development of a whole organism out of any part of sufficient size. Another test, more difficult and less frequently tried, is the rearing of a single organism from two eggs or embryos which have been made to fuse together.[11]

The experiments described in the foregoing section have shown that any half of the limb bud can give rise to a whole limb. Those to be taken up now demonstrate that two limb buds fused together will develop into a normal single limb.

The operation of superimposition or fusing together of two limb buds is carried out as follows: A circular incision of the proper size (3½ segments in diameter) is made through the ectoderm of the fore-limb region, care being taken to injure the underlying mesoderm as little as possible. The ectoderm may then be readily stripped from the middle layer by inserting the points of the scissors or needle at the dorsal part of the cut and tearing the ectoderm away. Often a few mesoderm cells, especially from the ventro-posterior quadrant, come off with the ectoderm, but the greater part of the cells composing the limb bud remain in place and not infrequently every cell is left intact. An entire limb bud from another embryo is then grafted in the usual way over the mesoderm of the limb thus exposed, and such grafts heal in very readily. The results of these experiments differ according to the orientation of the grafted bud, in harmony with the rules of laterality (Harrison, '17). At present only the cases in which the grafted bud has its normal orientation will be considered.

Five such experiments were made, all of the embryos surviving and giving the same results. Normal limbs developed which at first showed difference in size, but this difference was after a time obliterated. The greater massiveness of the double bud was usually apparent the day after operation and was most marked about three or four days later. In two cases it is recorded as persisting for twelve days, though the difference from the normal gradually diminishes, disappearing entirely by the time the yolk is entirely gone, i.e., about eighteen days.

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[11] Cf. Morgan, '95; Driesch, '00, '10; Goldfarb, '14.

It is clear from these experiments, even though small in number, that a single perfectly normal limb will regularly develop out of two limb buds fused together.

EFFECT OF REMOVING THE ECTODERM ALONE

In the early days of the study of the limbs the question whether the first sign of their appearance was in ectoderm or mesoderm was much discussed as one of phylogenetic significance. Whatever our attitude toward such questions may now be, it is important from the standpoint of developmental mechanics to inquire whether the factor which determines the development of the limb is located in the outer or the middle germ layer.[12]

Comparison of the experiments with cleaned and with uncleaned wounds, in which the ectoderm is treated the same way in both series while the mesoderm is differently handled, already suggests what the answer will be. The experiments described in this and the following sections are intended to add further and more direct evidence that the power to produce the limb rests primarily in the mesoderm.

It is not always possible to avoid injuring the mesoderm to a slight extent in the operation of removing the ectoderm. Therefore the experiments in which it was attempted to remove the ectoderm alone differ only in degree from the cases of simple extirpation with non-cleaned wounds. Five operations of this kind were performed, and in all five cases limbs developed promptly, though there was some delay as compared with the normal. The wound was not covered with grafted skin in any of the cases.

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[12] Braus ('09) has touched upon this question in connection with his study of the origin of the skleroblasts of the limb. On p. 165 he says, "In einer späteren Arbeit werde ich nachweisen können, dass in älteren Stadien ebenfalls das Ectoderm nicht für das Zustandekommen eines typischen Skelets notwendig ist; denn dasselbe bildet sich eben so gut unter einem ortsfremden Ectoderm, welches experimentell gegen die typische Nachbarepidermis ausgetauscht wird, und in grossen Entfernung von jeglicher Haut wie in der ungestörter Entwicklung." The paper dealing especially with this work (Braus, '08) is unfortunately not accessible to me at present.

EFFECT OF REMOVAL OF MESODERM ALONE

In order to remove the mesoderm alone, the ectoderm covering the limb region is first incised around three-fourths of its circumference. It is carefully lifted from the underlying mesoderm and left hanging by its ventral border. The mesoderm is then removed from the region below the pronephros, all loose cells being cleaned off, as in experiments already described, and then the covering layer is finally stretched back into place and held for a short time by a glass bar. The ectoderm contracts considerably while the wound is being cleaned, but with the aid of a fine needle it can usually be drawn over the wound. In some cases perfect healing was obtained in less than an hour. In others small areas of yolk were found still uncovered on the day after the operation. The quickness of the healing seems, however, to have no effect on the result, for in the three cases in which regeneration occurred, the healing was characterized as good, fair, and bad, respectively, while the cases of non-regeneration followed both good and fair healing.

Twelve experiments were made, in two of which the embryos died. In the ten remaining cases the wounds were of different sizes, varying from three to four segments in diameter, bounded as in the simple extirpations (p. 422). The results are given in table 8.

In five cases the cleaned area was of the smallest size, extending from the line between the second and third somites to that between the fifth and sixth. Three of these gave rise to regenerated limbs, while the other two did not. None of the other cases, which had larger wounds, regenerated.[13] In six of the cases which showed no regeneration the pronephros was left intact and in only one case was it removed. These experiments differ from those described in the third section (p. 432) only with respect to the region from which the ectoderm covering the wound is taken. The results are in full agreement, and the corresponding figures given in table 4 and table 8 could with propriety be combined.

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[13] In my preliminary note (Harrison, '15), on the fifth line from the bottom of p. 542, the words 'over three' should be substituted for 'four.'

TABLE 8

Showing effect of removal of limb mesderm

SIZE OF WOUND	CASES REGENERATED				CASES NOT REGENERATED			
	Healing of wound			Total	Healing of wound			Total
	Good	Fair	Poor		Good	Fair	Poor	
3 somites	1	1	1	3	2			2
3¼* somites				0		1		1
3½ somites				0	1	1		2
4 somites				0		2		2
Total	1	1	1	3	3	4		7

* In the table previously published (Harrison, '15, p. 540) in the first column fifth line read 3¼ instead of 3½.

While the number of cases is not large, it is beyond doubt that the presence of the normal ectoderm over the denuded limb region no more incites the development of a limb than does the presence of ectoderm from a distant region.

TRANSPLANTATION OF THE MESODERM

Transplantation of the whole limb bud, ectoderm and mesoderm, results, as is well known, in the development of a limb in the new position. Transplantation of one or the other of the two layers should afford additional evidence, more cogent than that already given, as to the potency of the several layers in determining the development of the appendage. Only one of these experiments, the transplantation of the mesoderm, has been tried; the negative results recorded with reference to the ectoderm in the previous section are thought to be sufficient evidence from that side.

In order to transplant or inoculate the mesoderm into some other region of the body, a pocket is first made under the skin of the embryo by sticking the points of a pair of fine scissors obliquely through the ectoderm and slightly opening them. The position chosen for this pocket was in most cases the flank of the embryo at the lower border of the muscle plates. In four cases, all of which resulted negatively, it was made above the eye. After the first embryo has been prepared, the ectoderm is removed from the limb region of another specimen, with as little injury as possible to the mesoderm, and the latter is cut out from below and behind the pronephros and transferred as a single piece to the pocket. It is often difficult to get this small mass of tissue inserted, because it is very sticky and is liable to be pulled out when the instrument is withdrawn. Having a small hole in the distal side of the pocket facilitates a deep insertion and consequently the retention of the transplanted cells. Healing of wounds of this character is rapid and without secondary complications. The mesoderm cells are, however, rather loosely held together and a considerable amount of disintegration may occur—more than when the limb bud is transplanted in toto.

The results of the experiments were as follows:

	cases
Embryo died prematurely	4
Resorption of transplanted tissue	5
Small nodule developed	3
Long appendage without digits	1
Limb of approximately full size with digits, usually showing reduplication	6
Total	19

These results are not essentially different from those obtained when the whole limb bud is transplanted, except that the limbs which do develop when the mesoderm is taken alone are more likely to show deformities. This was to have been expected in view of the difficulties of handling the mesoderm without the firm ectodermal covering to hold it together. Since the individual cases are of interest their histories will be presented separately.

Experiment Tr. Mes. 1. May 9, 1912. Mesoderm from left limb bud transplanted to left side. Some oozing from wound three hours afterward, indicating loss of tissue.

May 12. Small lump in region of graft.

May 15. Transplanted limb is getting much longer and is not much below the normal one in size.

May 18. Beginning to show digitations.

May 21. Larva has marked spinal curvature, but seems otherwise healthy. Transplanted limb has two well-marked digits and beginning of third. Limb not so long or so far advanced as the normal one. Good circulation.

May 25. The specimen was preserved on account of the deformity of its back, which rendered its existence precarious (fig. 38).

Transpanted limb is a left, having preserved its original laterality. It has a distinct third digit and the beginning of fourth, and on the radial side there is a reduplication of digits. The whole hand is, therefore, nearly symmetrical. Transverse sections show a small coracoid and a very shallow glenoid fossa, but the scapular portion of the girdle is not developed. Differentiated muscle fibers are present in the limb, but no nerves have been found.

Experiment Tr. Mes. 4. May 11, 1912. Right limb mesoderm to right side.

May 15. Grafted tissue not very prominent.

May 21. Limb bud 'points' anteriorly.

May 27. Digitations beginning; dorso-ventral doubling.

June 7. Specimen preserved.

The arm as a whole is a left, i.e., its laterality has been reversed (figs. 39 and 40). The radial digit is reduplicated on the radial border. There is another reduplication consisting of a long digit and a nodule, mirrored from an ulno-palmar plane.

In the normal limb on this side the first two digits are syndactylous, the first being short.

Experiment Tr. Mes. 5. May 11, 1912. Mesoderm from right limb bud implanted on right flank.

May 12. Wound still slightly open; transplanted tissue a good hump.

May 21. Transplanted tissue has grown and points more distinctly anteriorly.

May 27. Good circulation; two digits show; limb looks to be of normal form.

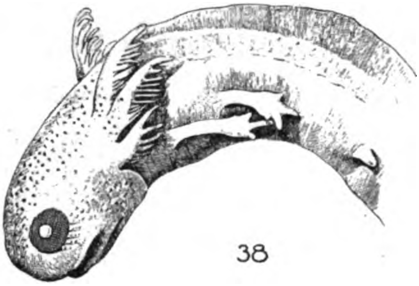
June 7. Specimen preserved.

Three well-marked digits are present with trace of fourth. The first two digits are webbed; otherwise the limb is normal (fig. 41). It is clearly a left, its original laterality having been reversed. There was no evidence of motility of the implanted limb before killing.

The preserved specimen was cut into frontal sections, examination of which shows that the shoulder-girdle cartilage is fairly well formed; the ventral (coracoid) portion is more extensive than the scapula, which is only slightly developed. Pronephric tubules, seen near the base of the limb, indicate that part of the pronephros was transplanted with the limb cells. Muscle tissue is well developed in the limb, though no nerve fibers seem to be present.

Experiment Tr. Mes. 16. May 13, 1914. Left limb mesoderm transplanted to right side.

May 14. Perfectly healed; small nodule caused by transplanted tissue.



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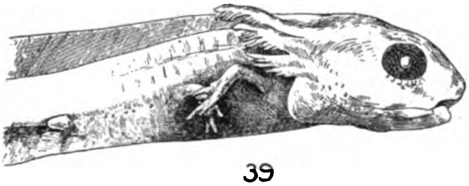

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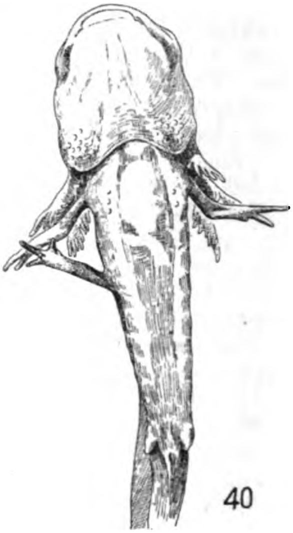

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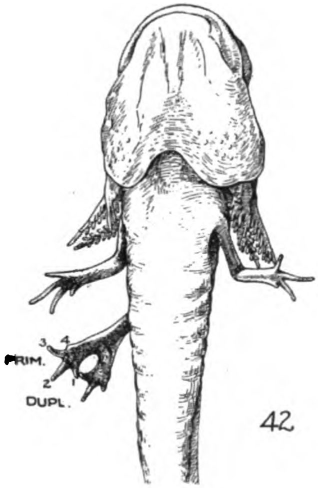

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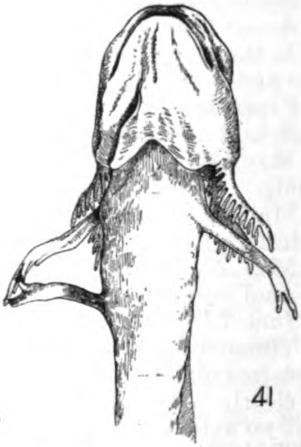

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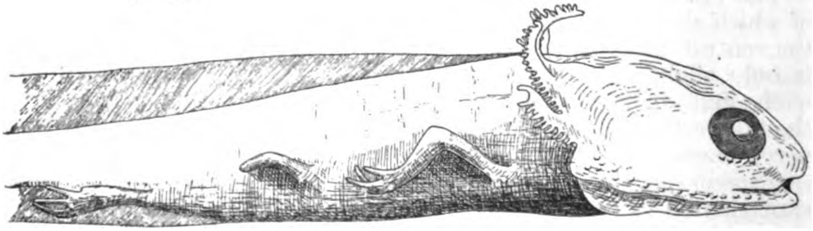

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May 20. Transplanted tissue growing well, 'points' anteriorly.

May 22. Growth considerable; reduplication beginning near base.

May 25. Limb consists of two almost equal parts branching near base; anterior member is bidigitate.

June 1. A perfect limb with reduplication of forearm and manus. Both members are tridigitate, with indications of fourth digit (fig. 42). The anterior member (*PRIM.*) which is the primary one, is a perfect left (original laterality of the tissue), the other (*DUPL.*) a right.

June 6. Larva preserved.

The specimen was cut into serial sections. The coracoid portion of the shoulder girdle is well developed and the glenoid fossa is marked. Dorsal to the joint there is no cartilage. Well-developed muscles run from the coracoid to the humerus, but the upper arm is almost devoid of muscle fibers. On the other hand, the forearm muscles are well developed. No nerve fibers have been found,

Experiment Tr. Mes. 17. May 19, 1914. Mesoderm of left forelimb to right side.

May 25. Transplanted bud sticks out almost straight from side of body.

June 1. Limb has grown considerably, but will probably be defective as to digits.

June 6. Unchanged. Limb cut off just below elbow.

June 29. Larva has grown well. Limb healed, but no hand has developed. It shows the elbow bend and has the posture of a normal right limb (fig. 43).

Experiment Tr. Mes. 18. May 19, 1914. Mesoderm of left limb-bud to right side.

May 20. Healing perfect; transplanted tissue a good nodule.

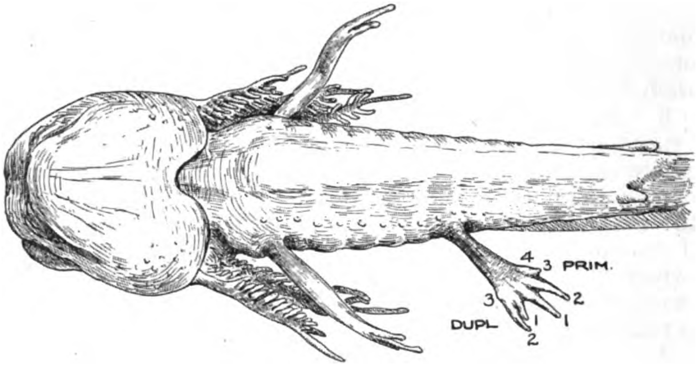
May 22. Nodule increasing markedly.

May 25. Transplanted tissue growing and points toward tail.

Figs. 38 to 43 Amblystoma larvae showing supernumerary limbs developed from inoculated mesoderm cells of the limb bud. × 7½. Figure 38. Experiment Tr. Mes. 1; the supernumerary limb is primarily a left (from left-handed tissue), but the hand is symmetrically reduplicated; sixteen days after operation. Figure 39. Experiment Tr. Mes. 4; supernumerary limb, primarily a left, though the inoculated tissue was from the right side; irregularities in the digits; twenty-seven days after operation. Figure 40. Same experiment, ventral view. Figure 41. Experiment Tr. Mes. 5; the supernumerary limb is a nearly normal left developed on the right side of body from tissue of right side. The only abnormality is the syndactyly of the first two digits; twenty-seven days after operation. Figure 42. Experiment Tr. Mes. 16. Ventral view of larva showing supernumerary limb reduplicated from elbow down; *PRIM.*, primary member, a left (from left handed tissue); *DUPL.*, secondary member, a right; twenty-four days after operation. Figure 43. Experiment Tr. Mes. 17. Supernumerary limb regenerated after amputation, a right (from left-handed tissue) with abortive hand; forty-one days after inoculation, twenty-three days after amputation.

May 27. The limb is bidigitate or bifurcated.
June 1. Limb is short, hand reduplicated and digits irregular.
June 6. Specimen preserved.

The double hand (fig. 44) is approximately symmetrical. The posterior member (*PRIM.*), which, judging by the first direction of pointing, is the primary one, is a right, the original laterality having been reversed. The first two digits are normal, the third is distinct, and the fourth a slight eminence. The other member (*DUPL.*) is not so far developed. The first two digits are webbed and the first consider-



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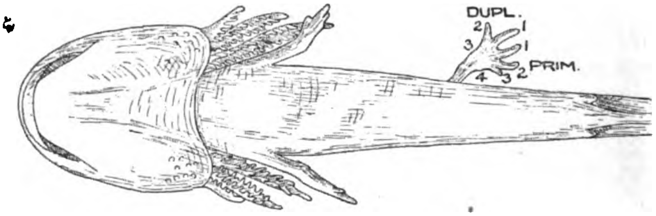

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Figs. 44 and 45 Larvae with supernumerary limbs developed from inoculated mesoderm (continued). × 7½. Figure 44. Experiment Tr. Mes. 18. Supernumerary limb with double hand; *PRIM.*, primary member, a right, developed from left-handed mesoderm; *DUPL.*, reduplicating members; eighteen days after operation. Figure 45. Experiment Tr. Mes. 19. Supernumerary limb with double hand; *PRIM.*, primary member a left, developed from right-handed mesoderm; *DUPL.*, reduplicating members; forty days after operation (development very slow).

ably shorter than normal. Examination of frontal sections shows that only the coracoid portion of the girdle is developed. Shoulder muscles are present, but no nerves have been found. There are two ulnae, one on each side of the somewhat thickened radius.

Experiment Tr. Mes. 19. March 3, 1915. Mesoderm of right limb to left side.

March 16. Growth of transplanted tissue has been comparatively slow, though in the past three days considerable.

March 19. Limb still short, though showing beginning of digitations.

March 29. Arm very short; three long digits, of which the most ventral and posterior is probably a reduplication. On the dorso-anterior border the third and fourth digits show, the latter but very slightly.

April 12. Specimen preserved.

This is similar to the previous case (Tr. Mes. 18), but the limb is shorter and the elbow bend more distinct. There is an approximately symmetrical radial reduplication of the hand, which is broad and paddle-like. The arm points posteriorly and laterally: The posterior member is probably the primary one and has had its laterality reversed, being a left. The digits are further developed in this hand, both the third and fourth being distinguishable (Fig. 45).

In viewing the above seven cases as a whole, it is seen that, while no one is absolutely normal, they are all sufficiently developed to show their specific form. One alone is badly deficient, two show reduplications which are slight, three others reduplications which are more marked, involving in one case the whole forearm and hand. One case (Tr. Mes. 5) has no reduplication and is normal except that the first two digits are held together by a web throughout their length. This abnormality is, however, not infrequently found in limbs that have not been operated upon.

These results show clearly that the specific limb-forming tissue is the mesoderm of a certain region of the body wall and not the ectoderm. When it is considered that there is much loss of tissue in the operation and that what remains must often be to a great extent mixed up, the most striking thing is that the limbs which develop from such inoculations should be so nearly normal. These experiments thus afford additional evidence for the equipotentiality of the elements constituting the limb bud.

No attempt was made to orient the engrafted tissue, if indeed this were possible, but the results which have been obtained may be interpreted in accordance with the results from transplanting the whole limb bud, where the orientation of the graft is known (Harrison, '17). In three of the cases the limb tissue was grafted on the same side of the embryonic body; one of these yielded a limb of the same side, while in the other two the laterality was reversed. In four cases the tissue was implanted on the opposite side of the embryo; in one of these the limb preserved the original laterality of the tissue, while in the remaining three reversal occurred.

CONCLUSION

The purpose and results of each of the experiments having already been pointed out, it remains only to state briefly their significance as a whole.

The tissue which is destined normally to form the fore limb has been delimited, and within the period of development dealt with in the experiments, it has been shown to be a self-differentiating system. It is a group of mesoderm cells formed as a proliferation of the somatopleure, and no specific stimulus from any particular portion of the ectoderm is necessary for its development. The exact boundary of the embryonic tissue which normally enters into the limb cannot be determined by the present methods; for, surounding the group of cells which constitutes the limb bud, there is a zone of mesodermal tissue, which, in case of removal of the original limb rudiment, may move in and assume the character of the excised material, giving rise after a time to a normal limb, as was first shown by Miss Byrnes ('98 b) in the case of Rana embryos. The limb rudiment may be thus regarded not as a definitely circumscribed area, like a stone in a mosaic, but as a center of differentiation in which the intensity of the process gradually diminishes as the distance from the center increases until it passes away into an indifferent region. Many other systems, such as the nose, ear, hypophysis, gills, seem to have the same indefinite boundaries which may even overlap one another.

Self differentiating as is the system as a whole, the parts within the system do not constitute a developmental mosaic, with the exception of certain portions of the shoulder girdle. The system itself is equipotential, as shown by the two tests to which it can be subjected; a whole will develop out of a part, and a single normal whole will develop out of two separate rudiments when fused together. The experiments of extirpating half buds and of superimposing buds, respectively, make this clear.

The limb rudiment, therefore, is an entity, which, except for its dependence for nourishment, is independent of its surroundings in the attainment of its specific form. In one important respect it has been found to be influenced by its position in the organism as a whole, and that is as regards its relations of symmetry and its power to form reduplications. The study of these phenomena will be the subject of a continuation of the present work.

SUMMARY

The fore limb of Amblystoma punctatum develops normally as a thickening of the somatopleure centering in the area ventral to the fourth myotome and extending over into the regions ventral to the third and fifth.

Numerous mitoses indicate that the growth of the limb bud is due to rapid proliferation of the cells in situ rather than to inwandering from surrounding territory.

Extirpation of the tissues of this region results in disturbances of development in the limb, the intensity of which depends upon the size of the wound, the care with which it is cleaned of mesoderm cells, and whether it is covered with grafted ectoderm or not.

If the wound is small or not entirely cleaned of mesoderm, a normal limb usually develops (regenerates) with some delay. After more radical operations the limb regenerates much less frequently.

When nothing further is done beyond excising the circular disc of tissue constituting the limb rudiment, the limb has been found to develop subsequently in 81.4 per cent of the cases (table 1). When all scattering mesoderm cells are removed, the limb develops in but 36.5 per cent of the cases.

Covering of the wound with ectoderm from the flank further reduces the probability of regeneration. For wounds of 3 somites in diameter the percentage of positive cases is reduced from 52 to 33, and development is entirely prevented by covering wounds of 3½ somites or over.

Even when the free appendage fails to develop, parts of the shoulder girdle are formed.

It is concluded that in these operations the cells that normally give rise to the limb are removed. In the subsequent process of wound healing cells in the surrounding zone move in and ultimately form a new limb bud. Around the limb-forming cells there is thus a zone of tissue which has the power, in gradually diminishing intensity toward the periphery, to form a limb vicariously.

When half of the limb bud is removed, disturbances of development may occur, but in many cases normal development of the limb follows.

These disturbances vary all the way from complete suppression of development to slight retardation. Abnormalities, such as defective digits or hand and reduplications, also occur.

There is no distinct correlation between the part of the limb bud removed and any particular defect.

Reduplications are most frequent after extirpation of the anterior half.

Normal development may occur after excision of any half of the limb, but is more frequent in case of removal of the ventral half.

The limb-forming material is divided into approximately equal parts by lines anterior and dorsal, respectively, to the vertical and horizontal diameters of the limb disc 3½ somites in diameter (fig. 2).

On the whole, these experiments show that, while quantitatively the limb-forming tissue is unequally distributed in the four quadrants of the area usually extirpated, there is no qualitative difference in the potencies of the cells of the four quadrants.

Splitting of the limb bud by a vertical or horizontal incision may cause slight delay in development, but does not produce reduplications.

A single normal limb will develop from two fused limb buds when one is superimposed upon the other in proper orientation.

Removal of the ectoderm covering the limb bud has no effect upon the development of the limb beyond causing slight delay.

Removal of the mesoderm of the limb bud, when complete, prevents the development of the limb even when the proper ectodermal covering is healed back over the wound.

Transplantation (inoculation) of mesoderm from the limb region to a pocket under the skin of the flank results in many cases in the development of limbs. In about half the cases which live this inoculated tissue is resorbed or does not develop further.

The limbs which develop show a large proportion of reduplications; one case was perfectly normal except for syndactyly of the first two digits.

Taken together, the experiments show that while the ectodermal covering is indifferent, the mesoderm of the limb bud constitutes a specific self-differentiating system, which, however, in itself is an equipotential system in Driesch's sense.

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EXPERIMENTS ON THE PHYSIOLOGY OF DIGESTION IN THE BLATTIDAE

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TWENTY-ONE FIGURES

INTRODUCTION

The following experiments were undertaken to explain certain phases of digestion in cockroaches, and especially the processes concerned in digestion of fat, as well as other related problems. The subject is not a new one; many investigators have studied various phases of the problem, but they have in several cases arrived at very different conclusions, One of these workers was Prof. Alexander Petrunkevitch ('00), who suggested the problem to me.[1]

The real object of my investigation has been to attack the problem from a new point of view, besides repeating to a greater or less degree the experiments of previous workers. I have endeavored to amplify considerably the scope of previous work through the use of newer stains and the physiological and experimental methods.

The main problems which I hoped to investigate and elucidate were concerned with the rôle of the crop in digesting and absorbing food, the various functions of the gizzard, the details and significance of digestive processes in the stomach and coeca, and the explanation of the presence of certain substances and leucocytes in the tracheal tubes.

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[1] Prof. Petrunkevitch's paper appeared in 1900, and since then several investigators have come to results distinctly at variance with his. This disagreement is explained in part by the fact that Professor Petrunkevitch gave almost no data concerning the details of his experiments. Most of the data were lost in consequence of political disturbances, and without the data the work could not be defended. Moreover, lack of these data prevented repetition and verification of the experiments. After many trials I arrived at methods of experimentation which tended to verify Petrunkevitch's results, and to explain the disagreement of other authors. From this beginning I proceeded to further and more varied experiments, always using groups of animals in each experiment. More than six hundred cockroaches were used in all.

MATERIAL AND METHODS

The material first used consisted in the species of cockroach previously used by most earlier investigators in the field of fat digestion in insects, namely, Blatta orientalis Linnaeus (Periplaneta orientalis), the common black cockroach, and Blatella (Philodromia) germanica, the croton bug. The latter species was found rather small for convenient dissection and observation. Later on I used Periplaneta americana, the American cockroach, almost exclusively. In this species, which is very hardy and much larger, it seemed hopeful that some processes which had been obscure or disputed in the smaller species might be seen more plainly, and indeed this proved to be the case. One especially fortunate fact was discovered, namely, that the body cavity in the abdominal region could be entered in the living animal by turning back a flap of the body wall. After the flap was replaced, the animal experienced no serious inconvenience. It was found possible thus to make ligations of the alimentary canal just after food was taken, thus isolating certain digestive organs from connection with others. This eliminated the uncertainty of previous work as to the flowing of digestive juices from one part to another. The digestion which occurred in a part tightly ligated from other parts was considered to be surely produced by digestive fluids and enzymes secreted in that part.

Another fortunate feature about Periplaneta americana is its tolerance of sunlight. Unlike Blatta orientalis, which is very sensitive to light and avoids it whenever possible, my animals walked around contentedly even in bright light and took food normally.

The tissues were usually fixed in Flemming's strong solution, which contains osmic acid and stains fats black by an oxidative process. This fixing fluid is especially effective in work with olive oil, for the pure oil consists mostly of the glyceride of oleic acid, and oleic acid is the only one of the four common fatty acids of ordinary fats which is blackened well with osmic acid, this being due to its unsaturated molecular structure. In work with tissues containing blackened fat it seemed inadvisable to stain with any haematoxylin stains, for these stain so darkly and tend to obscure appearances of the darkly stained fat. As a general stain acid fuchsin was used successfully; it stains nuclear and cytoplasmic structures sufficiently distinctly. For obtaining the best histological details Perenyi's fluid was much used, and also a mixture of 5 per cent formol and 50 per cent alcohol. The best combination of stains for accurate definition of structures was acid fuchsin, followed by Ehrlich's haematoxylin. In working with the very compact and hard tissues of the gizzard, Flemming's killing fluid was very satisfactory when it penetrated well, which was not always the case. Perenyi's fluid was also satisfactory. In order to bring out the muscle structures of the gizzard, careful staining with iron haematoxylin and picrofuchsin is necessary. When the animals had eaten fat stained with Sudan III or Nile blue sulphate, sections were made in a freezing microtome.

Most of the tissues contain so much water that extra care is necessary in running them through a series of reagents. In running ordinary tissues from absolute alcohol through xylol to melted paraffin, it is very desirable to interpose between the alcohol and xylol two appropriate mixtures of these reagents. In running from xylol to melted paraffin, it is necessary to interpose a cold and a warm mixture of the two. Unless this is done, the nuclei and cytoplasm shrivel and the fat globules become distorted and liable to fade rapidly.

The fat used as food in the experiments was pure olive oil. The animals refused to take pieces of solid fat, but would take bread soaked in olive oil. But the ideal food seemed to be a paste of pulverized sugar and olive oil. This was taken greedily, even when stained brightly with Sudan III, Nile blue sulphate, or litmus powder. The organs chiefly concerned in recognizing the food semed to be the maxillary and labial palpi, not the eyes or antennae. The feeding was done in covered glass jars, in one part of which some of the paste was placed. The animals, when put into the jars, walked or ran about until the mouth parts and palpi happened to touch the food; then stopped, moved the palpi over the food and began to eat by opening and closing the mandibles. After feeding was completed the animals were removed to a clean dish. When colored foods had been eaten, it was always possible to decide in a general way how much a given animal had eaten, for the color of the food in the crop showed through the transparent crop wall and the semitransparent region of the body wall between the third pair of legs. When an animal had eaten a moderate or very generous amount of the paste, it stopped to clean itself carefully, going over the whole of the anterior legs with the mouth parts, and drawing the antennae through the mouth parts by aid of the anterior legs, meanwhile apparently licking off any oil or other extraneous matter on them. The fat-feeding methods of Schlüter were also employed; that is, the smearing of the head and anterior body with olive oil with a brush and then relying on the cleaning habits of the animals to get the food into the mouth and crop. This method was considered inadequate and not practiced much, because it was so unnatural, and because experience showed that fat smeared on the thorax entered the thoracic spiracles readily, and thus gave false pictures of fat distribution in subsequent preparations.

ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL

In view of the fact that there is a discrepancy of opinion as to the structure of the alimentary canal, it seemed desirable to investigate it once more without going into unnecessary detail. This was undertaken as a basis for physiological work. The comparative lengths of the various parts in a specimen where the total length of the alimentary canal was 6.7 were as follows: oesophagus 0.25 cm., crop 2.5 cm., gizzard 0.25 cm., stomach 1.3 cm., small intestine 0.25 cm., large intestine 1.6 cm., rectum 0.5 cm. It will be noted that the crop possesses by far the largest surface of any single organ, being 2.5 cm. in length and on the average 0.6 cm. in diameter when distended with food, thus having a surface of about 3 sq. cm. as against a corresponding surface of 0.7 sq. cm. in the stomach, which is 1.3 cm. in length and 0.25 cm. in diameter.

The stomodeum, or that part of the anterior alimentary canal which is turned in during the embryonic period, is of considerable size in the American cockroach, and may be divided into six parts: mouth cavity, pharynx, oesophagus, crop, anterior gizzard, and posterior gizzard. The mouth cavity and pharynx were not studied, for it is most improbable that any important digestive changes occur there. The salivary glands are paired, one lying on each side of the crop along about half its length. The ducts run forward into the mouth and are strengthened by a tough spiral structure similar to that of the tracheal tubes. The reservoir of the salivary secretion may be very large, it may be almost as long as the crop when it is dilated with fluid.

Oesophagus. The oesophagus begins where the dilation of the pharynx ends and varies much in size according as it is dilated with food or not. When empty its cavity is nearly obliterated by closely appressed folds. These folds are continuous with those of the crop to such an extent that it is impossible to say where one ends and the other begins. The oesophagus wall consists of a single layer of nearly cubical epithelial cells (fig. 1), between which are seen muscle processes and tracheal end cells similar to those described by Petrunkevitch. Within the layer of cells and secreted by it is the chitinous intima, of about the same thickness as the cells. This is raised into projections, on the summits of which long bristle-like processes are situated. Around these structures is a layer of circular muscles.

Crop. The crop is by far the largest part of the alimentary canal and no doubt has an important function, though Schlüter considers it as a mere storage organ and conducting tube to the stomach. The wall consists of three layers, muscular, epithelial, and chitinous. The muscular layer consists of two layers of striated fibers at right angles to each other. One layer consists of circular muscles and serves to contract the crop walls tightly over any food within. The other layer consists of longitudinal muscles. The muscles form a loose network, and through the meshes the general body cavity connects with spaces under the epithelial cells. These spaces are under folds in the epithelial layer in the usual state, but are nearly or quite obliterated when the crop is distended with food. In these folds are found muscle fibers and processes which run from the muscular layer to the epithelial and chitinous layers, and may be considered as radial muscles, as shown in figure 2. Wandering blood cells and tracheal branches are also found in these spaces (fig. 2). The latter extend on one side to larger tracheal branches and on the inner side to tracheal end cells.

The epithelial cells have the shape of hexagonal prisms twice to four times as high as broad. The nuclei are situated near the bases of the cells; that is, toward the body cavity, as shown in figures 2, 5, 17. The cytoplasm of the nuclear region usually stains more deeply than the cytoplasm of the other end of the cell, and is apparently more specialized. Between the cells and the lumen of the crop is the rather thin chitinous intima. Petrunkevitch has demonstrated that this intima is porous, and I have verified his work, using his methods. Some earlier workers used this porosity to account for the passage of fat globules into the cells from the lumen, but it is now known that fats cannot be absorbed as such, but must be split to fatty acid and glycerol, both of which, being soluble, may be absorbed into the cells.

The muscular layer may be separated to a greater or less extent from the epithelial layer by teasing with needles under a binocular microscope. This applies for the oriental cockroach, and not for the American cockroach. Flat preparations of the epithelium may be made by holding a piece of the crop's wall tightly down and flattening out folds under a coverglass, then running in Flemming's fluid, or absolute alcohol, as recommended by Petrunkevitch. A much better method of getting flat preparations consists in feeding an animal until its crop is distended and therefore all the folds of the surface are smoothed; the crop is then removed without puncturing and preserved in

Perenyi's fluid. In the American cockroach there is a definite region of the anterior crop where there are very few muscles, this region is especially favorable for study. In these preparations the epithelial cells appear mostly as hexagons.

Petrunkevitch has described certain unusual conditions in some of these cells, and has figured them as seen when flat and in cross sections. In some cases the cell walls of a group of cells degenerate and the cells fuse. The cytoplasm appears vacuolated and abnormal and the nuclei begin to degenerate. Finally the whole mass of protoplasm seems to be discharged and the gap filled by the growth and cell divisions of adjacent cells. He also describes cells which contain two or more nuclei, but do not appear abnormal otherwise; these represent small syncytia resulting from nuclear divisions with suppressed cytoplasmic divisions.

Among my preparations some slides show stages which may be likened to these. The cells show great inequality of size, the very small ones appear to be latent, but ready to replace by growth any gaps in the epithelium. Binucleate and trinucleate cells often appear, but I cannot explain their significance, perhaps they represent small syncytia. Some of them are shown in figure 18. Another unusual condition is shown in figures 17 and 18. In such cases the cytoplasm appears abnormal and numerous nuclei are present, some of them appearing degenerate or as mere vesicles. No traces of cell walls are present in these regions, and the cytoplasm seems dead. I have been unable to find a definite explanation of such pictures; perhaps they represent a sort of lesion which results from injury to the cells through mechanical damage by sharp pieces of food or through the action of poisonous matter in the food. The process cannot, I think, be compared with the normal casting of cells of the stomach epithelium after they have become worn out by continual functioning.

Petrunkevitch has also figured ring cells; that is, cells which contain a very large vacuole surrounded by a thin layer of cytoplasm containing the nucleus. He believed that these cells represented a stage in the fusion and casting of cells, especially as two or three cells might combine to one ring cell. In the species I have studied this appears not to be the case. I have never found any such appearances except in preparations which were fixed in absolute alcohol, and even then the appearances were not distinct, and probably represented artifacts due to too rapid dehydration.

Gizzard. The gizzard consists of two distinct parts, an anterior and a posterior. Its shape is in general that of a cone whose blunt end is anterior and adjoins the crop and whose pointed end extends into the stomach. Its altitude about equals the diameter of the base. The hinder, protruding part is of about the same length as the anterior part. The two parts are entirely different histologically. In the anterior part the chitin forms six heavy teeth; the chitin has no distinguishable structure for the most part, but may be shown by staining with erythrosin to consist of three homogeneous layers, an outer and an inner layer which take no stain and a median layer between them which stains bright red.

The six thick teeth fill most of the cavity of the anterior part. They are large projections, triangular in cross section and roughly rectangular in longitudinal section, which nearly fill the space within, leaving only a narrow lumen in the middle and small spaces between their sides (fig. 8). The outlines of the teeth are not quite triangular, but often have swellings or concavities on the sides and somewhat flattened points. Usually a swelling of one tooth lies opposite a cavity on the adjacent tooth, and flattened and pointed ends do not sharply oppose each other. Ramme has found that in cockroaches the six teeth fit tightly and perfectly and are so well held together by the thick circular muscles that a tight fastening may be made.

Under the chitinous intima lies a single layer of long cylindrical epithelial cells, and below them a mass of connective tissue, through which run many tracheal tubes. Just under the epithelial cells these tubes end in tracheal end cells, whose processes run up between the cells above. Outside of the connective-tissue layer is a broad layer of circular striated muscles.

Between the main teeth are secondary and tertiary ones. These, unlike the primary ones, are covered with short spines. Between each pair of large teeth are three secondary ones, evenly spaced (fig. 9). Between each primary tooth and its adjacent secondary tooth are three or five tertiary teeth, and between adjacent secondary teeth are two or three tertiary ones. So the gizzard has six primary teeth, eighteen secondary ones, and sixty or ninety-six tertiary ones.

The teeth extend through about half the length of the anterior gizzard. The region between their posterior ends and the passage from the gizzard into the stomach is provided with smaller rounded projections which are set with long yellow spines or needles. Just behind the primary teeth are considerable folds, which Miall and Denny have called cushions for the teeth. These are shown in figures 8 and 9. The spines here are large and uniform, and are situated in sockets on the summits of dome-shaped chitinous projections. The structure and relations here will be discussed later. Posterior to the cushions lies a region of many smaller lobes and folds, the walls of which merge into the duct leading to the stomach. These lobes are thickly set with simply-structured bristles. Adjacent and opposite lobes are usually closely opposed to each other, so that little or no passage is left (figs. 8 and 15). They may thus effectually block the passage of food from the crop through the gizzard to the stomach.

The epithelium of the anterior part of the gizzard merges into that of the posterior. This posterior part projects far into the stomach and consists of one epithelial layer bounding the lumen and another which turns back from the apex of the projection parallel with the first and outside of it. This epithelium merges with the extreme anterior end of the stomach wall, in the region where the coeca originate. The lumen of the projecting part of the gizzard is small and is almost obliterated, being reduced to a star shape by the projecting rounded folds of epithelium, which are surrounded by muscles. Occasionally I have found small black bodies in the cells here in preparations fixed in Flemming. The epithelium of the outer side bears small rounded chitinous bodies to which short spines are attached.

Stomach and coeca. The stomach extends from the gizzard to the point of entrance of the Malphigian tubules. In its normal position it is curved, and bent so that its posterior end is almost directly below its anterior end. It is crowded in the abdominal cavity, and is small in proportion to the size of the animal and in proportion to the crop's size. From the anterior end arise eight coeca, each of which is of about a third the diameter of the stomach and of about half the stomach's length. The variation in size is very great, especially depending on whether the coeca are filled with food or not. Sometimes the coeca are as long as the stomach. The outer wall of the stomach is composed of a loose meshwork of muscular fibers, among which tracheae ramify. The same can be said for the outer wall of the coeca. The assertion of Jordan that the fibers of the muscle meshes only pass under unspecialized cells seems to apply in the American cockroach. In this way much of the surface is left free of striated muscle, which would, if present, hinder the passage of food materials from the epithelial cells into the body cavity.

The epithelial cells are in general long and narrow as seen in either transverse or longitudinal section, and are similar throughout the whole length of the stomach. As in most insects, the inner edge is set closely with fine filaments which serve to protect the epithelial surface (fig. 21). At very frequent intervals groups of immature cells are seen between the true epithelial cells and the muscular layer. These cells are small and closely packed. In many places all intermediate stages between them and adult cells are seen, as shown in figure 21, so it seems certain that there is a continual replacement of the mature cells, which often die and are cast, as I shall later describe. The epithelium usually shows but few adult cells in a group, and these groups more or less widely separated by the intermediate cells which take their places later. The surface of the epithelium may be flat or undulating, but is often raised into small villi with U-shaped spaces between them, the so-called crypts of Frenzel. The ends of the villi may be smoothly rounded or bulged out considerably. The arrangement in villi allows a much greater surface for secretion and absorption. I agree with Biedermann that the crypts are not glandular in function, but are adapted for efficient regeneration of cells. The membrane lining the wall of the epithelium is evidently cast at intervals, for it may be seen at various stages of disintegration. Such stages correspond to those described by Schroder. The spheres of secretion which appear on the surfaces of the cells will be discussed in a later section on the functions of the stomach.

The structure of the epithelium of the coeca is the same as that of the stomach in every detail. This shows plainly in sections which show the branching of the coeca from the stomach. In these cases it cannot be determined just where the coeca originate. I have seen practically no evidence of more secretion globules on the coeca epithelium, an observation which is surprising in view of the fact that several authors have described the coeca as preeminently secretory organs, capable of producing most of the secretion found in the stomach. Later I shall show that not only are the histological structures of the stomach and coeca alike, but also the digestive functions.

At the hinder end of the stomach the epithelium makes a circular fold which partially constricts the passage into the small intestine. This may be considered as a valve. Schroder suggests that the purpose of it is to help roll up boluses of food matter and pass these on to the small intestine, instead of a constant stream. Backflow is somewhat prevented, too.

Small intestine. The small intestine is very short and has a small lumen. Just behind the fold or valve at the hinder end of the stomach the high epithelium of this region gradually becomes lower and merges into the thin epithelium of the small intestine. At this point the Malphigian tubules enter in six great groups. The epithelium in this section consists of nearly cubical cells whose cell boundaries are hard to make out. The layer of cells is very much folded. The chitinous intima bears small swellings surmounted by very short spines. The cell structure resembles that of the oesophagus. Surrounding the epithelium is a thick layer of apparently confused muscular fibers. The function of the small intestine is not absorptive, and it has never been so considered except by Frenzel and Deegener. The explanation of their findings may lie in their considering cells immediately behind the stomach valve as intestinal cells, while they really belong to the stomach.

Colon. The large intestine or colon is long and coiled in its position in the body. Its epithelium consists of long, narrow cells which are thrown into rather regular folds. Under the folds tracheae and tracheal end cells may be seen. In the American cockroach the histological details may be seen well after using Flemming's strong killing fluid. On the contrary, in the oriental cockroach, the structure is spoiled by Flemming's fluid, as Petrunkevitch and Schlüter agree. In any case Perenyi's fluid is satisfactory. The colon is divided from the small intestine by a fold or valve of the epithelium. The intima is raised into significant mounds over each of the cells, but no hairs are evident, as described for Blatta orientalis. The function of the colon is not absorptive, at least I have not found it so, nor have many other authors except Frenzel, and his observations have since been disproved by later investigators. Simroth and Mingazzini have stated that certain sacs of the colon were absorptive in Lamellicorn larvae, but their descriptions indicate that there was no real absorption, but mere passage through pores. Berlese has described a similar case.

Rectum. The rectum is the last part of the digestive canal and terminates at the anus. It is surounded by a thin muscular layer. The so-called rectal glands are six lobes of very long cells which bulge far out into the lumen. Their cells do not quite reach the muscular layer, but leave small interspaces, in which tracheae ramify. The cells of the glands have a distinct intima, but no chitinous filaments. This intima is continuous between the glands and dips down almost to the muscular layer, there being almost no cells between.

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Oesophagus. The function of the oesophagus is merely to conduct food into the crop or to retain it for a time if the crop is quite full. The thick intima evidently precludes all secretion and absorption here. No globules of absorbed fat were ever seen in the cells.

Salivary glands. Various investigators have found that the reaction of the saliva in Orthoptera is never acid, but may be neutral, as Plateau found in Blatta orientalis, or alkaline, as in Blattella germanica. Jordan states that the saliva has about the same digestive power on carbohydrates that human saliva has.

Crop. If we consider the function of the crop, there are two principal possibilities, 1) secretion and 2) absorption. A third possibility is storage of food, and this is no doubt an important factor, enabling the cockroach to store here much of a very large meal and utilize it during a foodless period of several days or weeks, especially during bad weather in its native home in the tropics. The large size of the crop is evidently an important factor in making them as hardy and prolific as they are. This is in accord with Jordan's theory that the manner of life brings about more variation in the foregut of insects than in the mid and hind guts.

I have almost never found the crop entirely empty, though after three weeks of starvation in a clean glass jar it may contain nothing but a small amount of fluid. Under such conditions, they ingest various non-nutritious substances, such as wood shavings and tarsi and pieces of antennae of their mates. The content usually appears as a sticky gray mass. After they had eaten all they could of the red mass I fed them, their crops were very much distended, completely filling the thoracic cavity and more than half of the abdominal cavity, extending as far back as the fifth abdominal segment. The pressure caused the epithelium to bulge out between the muscle strands. Frequently this pressure was so great that a part of the food was regurgitated. Such a meal may last the cockroach two months or somewhat less, as found by dissection at various intervals. This surely shows that the crop is important as a storage organ.

The epithelial cells are all alike histologically and show no special characters of secretion or absorption. Experiments to be described herein showed that secretion occurred in these cells, but methods of direct observation proved insufficient to determine the reaction of this secretion. The reaction of the content of the crop depends largely on the kind of food in the lumen, partly on the secretion of the crop cells, somewhat on the secretion which flows in from the stomach, and partly on the secretion which the salivary glands have poured in. It is certain that in the crop the neutral or alkaline secretion of the salivary glands acts on carbohydrates and renders them soluble, as Jordan and others have established.

To obtain information as to the reaction of the secretion of the epithelium of the crop alone, I removed the crops from ten animals and placed them in normal salt solution, then slit them longitudinally, and carefully washed out the contents. The crops were then ground fine in a mortar with 10 cc. of clean salt solution, and the mixture left two hours to make an extract. The mixture was then filtered and the filtrate tested. This filtrate proved to be neutral to phenol-phthaleine and alkaline to litmus. This indicated definitely that the normal secretion of the crop is slightly alkaline.

Though the crop secretion is alkaline, I did not always find in my feeding experiments that the contents of the crop were alkaline after fatty food had been eaten for certain periods. Nile blue sulphate is a good indicator in work with fats, for it stains fats blue and fatty acids red. I fed an animal a large amount of a paste of olive oil and sugar, stained bright blue with the indicator stain. Three days later, on dissection, the whole content of the anterior crop proved to be deep red, while the epithelium was blue. The presence of red fatty acid in the lumen indicated that some agency, probably an enzyme, had acted on the fat to produce fatty acid. This process was more definitely explained by a series of feeding experiments in which powdered litmus was mixed with the food eaten by the animals. If, when the crop was subsequently opened, the contents were observed to be red, they were considered to be acid in reaction; if blue, alkaline.

Miall and Denny, Ramme, Jordan and Biedermann have maintained that the acid secretion found in the crop after feeding was derived entirely from the stomach and coeca, which had flowed from them through the gizzard into the crop. Their idea is that a large part of the digestion occurs in the crop, but that the enzymes came all originally from the stomach, coeca, and salivary glands.

My investigations indicate that the findings of the above investigators are partially correct, but not entirely so. The crucial experiment was the following. An almost starved roach was fed a large amount of a mixture of olive oil, powdered sugar, and blue litmus powder. Immediately afterward the abdominal cavity was opened and the crop tightly ligated just anterior to the gizzard. The operation was done carefully and without much inconvenience to the animal. The parts were replaced in as nearly natural conditions as possible, and the animal was left for twenty-four hours, then dissected in salt solution. The colors of the contents of the various parts were observed to be as follows: crop anterior to ligation, red and some blue merging into red; crop at ligation, blue; crop posterior to ligation, deep red and a little blue merging into red. The redness of the crop posterior to the ligation indicated, no doubt, that acid secretion had migrated thence from the stomach and through the gizzard. The distinct blueness at the ligation point indicated that the ligation had been successful, for if it had not been, the acid secretion of the stomach would have flowed in through the gap and the color would have been red instead of blue. The explanation of the redness anterior to the ligation is an interesting problem. Redness here means acidity, and the real question has to do with the origin of the acidity. Four explanations seems possible: 1) that some stomach secretion got into this part of the crop; 2) that the saliva from the salivary glands caused the acidity; 3) that the reaction of the food itself was acid, and 4) that the acidity was caused by the action of some secretion or enzyme of the crop itself. The first possibility need not be considered, for the tight and successful ligation, as mentioned above, prevented any flow from the stomach to this region. The second possibility is not valid, for all investigators hold that the saliva is alkaline or neutral. The third possibility is ruled out by the fact that some of the original paste was left in the feeding jar all through the experiment, and was as blue at the end as at the beginning. So the fourth possibility seems to be the true one, and the acidity must be considered as derived from secretion or enzyme action of the crop. If it is derived from a normal and usual acid-secretion flow, this secretion should be present after feeding of various foods. To test this a roach was fed a large amount of a paste of mineral oil, powdered sugar, and blue litmus powder, then the body cavity entered and a ligation made as before, and the animal left twenty-four hours before dissection. The experiment was thus exactly the same as the preceding except for the substitution of the indigestible mineral oil for the digestible olive oil. In this second specimen it was found on dissection that the crop posterior to the ligation contained deep red material just as it did in the first specimen; but the crop anterior to the ligation was completely blue. We must now explain why acidity occurs in the crop after olive-oil feeding, but not after mineral-oil feeding. All other possibilities having been ruled out, it seems that the acidity must have come from some product of the olive oil. This oil is neutral in reaction, but one of its digestive products is acid, namely, the fatty acid, in this case mainly oleic acid. This acid seems to me to be the only possible cause of the acidity observed, and if it is present it indicates that the fat (olive oil) has been split to its components in the crop and under the influence of the crop alone, other possible influences having been eliminated. The fat-splitting enzyme of the alimentary canal of animals is lipase. There seems good evidence that this lipase is secreted by the crop. Its purpose is to prepare the insoluble fats for use by changing or splitting them to soluble products, capable of diffusion.

The real test of a digestive process consists in digestions carried on outside of the body. This method also allows quantitative calculations and affords an actual and true comparison of the digestive power of various organs. It also gives an opportunity to decide whether Petrunkevitch was right in asserting that the crop is the most important organ in fat digestion or whether it is a mere subsidiary to the stomach, as so many authors have asserted. Trials show that artificial digestions may readily be carried on. The test of the amount of digestion consists in titration against alkali. When fat is digested fatty acid is produced, and the amount of acidity present at any stage depends on the fatty acid present, and is a measure of the extent to which fat has been split, or, in other words, how far its digestion has proceeded. Thirty animals were used in the crucial experiment. They had been starved for varying periods, so that little food was present in the crops or stomachs. All receptacles used were cleaned by boiling at the beginning of the experiment and physiological precautions were observed. The animals were all carefully dissected, the crops and stomachs removed, the crops put into one dish of normal salt solution, and the stomachs with their coeca into another. After the salivary glands were removed, the crops were slit longitudinally, and the contents carefully washed out with a soft brush. The thirty crops were rinsed and ground to a pulp in a mortar, 30 cc. of normal saline being added. This was left for two hours while the enzyme was extracted, then the solid matter was removed by filtration. The filtrate (about 25 cc.) was made up to 30 cc. with saline and divided to three portions of 10 cc. each in flasks. One portion was titrated at once against 1/20 normal NaOH, using phenolphthaleine as an indicator, and found to be neutral. The flask containing another portion of 10 cc. was plugged and left three days at room temperature in darkness, then titrated. This showed an acidity corresponding to 0.1 cc. of the alkali. This slight increase in acidity over the original amount was due to bacterial or other causes. To the third flask of 10 cc. of the extract was added 10 cc. of pure olive oil (neutral by titration tests), the mixture was left three days in darkness at room temperature and shaken at intervals. It then showed by titration an acidity corresponding to 10.8 cc. of 1/20 normal alkali. Comparing the flasks with and without olive oil, there has been an increase in acidity corresponding to 10.7 cc. of alkali. The two flasks contained the same amount of the same extract, and were treated similarly, but for the addition of the oil to one. Thus the acidity must have been derived from the olive oil, and must represent the presence of fatty acid as a product of the oil. The presence of fatty acid indicated that splitting of the fat had occurred during the experiment, or, in other words, that digestion of the fat had occurred outside of the body by the enzymes derived from the cells of the crop's wall.

The thirty stomachs with their coeca were in general treated like the thirty crops. Their contents were pressed out through the open ends, and the coeca were punctured when it was impossible to remove their contents otherwise. The grinding, extraction of the enzyme, filtration, and division to three portions of 10 cc. was performed as described for the crops. Immediate titration of one portion showed an acidity corresponding to 0.3 cc. of 1/20 normal alkali. The other two portions were left three days as before, oil being added to one. The portion without oil titrated against 1.0 cc. of alkali, showing an increase of 0.7 cc. due to bacterial or other action. The portion to which 10 cc. of oil had been added titrated against 7.2 cc. In this case, then, there was an increase in acidity of 6.2 cc. over the control. This increase must have been due to digestion and production of fatty acid, as just discussed for the crop. This is just what all investigators would anticipate, I think, for all have considered the stomach as an important organ for the preparation of food for the uses of the body.

The above results show without question that fat is digested in the crop and stomach. They also afford us comparative values for the importance of the digestion in the two organs. The figures indicate that the crop is more important than the stomach in the proportion 10.7 to 6.2. I shall later show that more than half of the effect of the stomach with its coeca is caused by the coeca, so the results really indicate that the crop is between three and four times as potent in fat digestion as the stomach per se. All question of individual variation is obviated by the fact that thirty animals were used together in the experiment. The crop is therefore the chief organ of digestion of fats, just as Petrunkevitch stated in 1900.

In other experiments some extracts of enzyme were boiled. These were compared as to digestive power with unboiled ones. Such boiled preparations showed much less acidity in later stages of fat digestion than unboiled ones. The fact that the active agent of the digestion is destroyed by boiling is additional evidence of its enzymatic nature.

I attempted to use the soluble fat ethyl butyrate in a series of experiments similar to those described. In every case the acidity at the end of the experiment was about the same as that at the beginning, so there was evidently something toxic about the fat which resisted the enzyme, as other workers have found in vertebrates.

Absorption in the crop is not a generally accepted view at the present day. No author of the last decade has spoken decidedly in its favor; nevertheless, I believe it regularly takes place and is of very large importance. Plateau was the first careful investigator in this field; he concluded that in the form he studied a large part of the absorption of food occurs in the crop. Jousset de Bellesme found similar results in his experiments with sugar, in which he seemed to prove that sugar entered the cells from the lumen by diffusion. Both of the latter investigators found evidence that starchy food does not soon pass into the stomach, but is retained by the crop to be digested and absorbed. In criticism, Cuénot and Biedermann have sought to disprove this by assuming that there is a continual passage into the stomach and subsequent rapid absorption; therefore but little is ever present in the stomach's lumen.

Cuénot observed that the cells of the crop have a chitinous intima, while those of the stomach do not, so he reasoned that absorption should only occur in the stomach. His conclusions were not entirely verified by experimental work, so they cannot be accepted unreservedly. He fed colored fluids to Blatta, and found them after several days present in the stomach only. I have never been able to repeat this experiment, and I believe we must conclude that some of the colored matter he fed was absorbed by way of the crop during the several days the experiment lasted, so none was present there when the animal was dissected.

Petrunkevitch in 1898 made a series of experiments in which he starved cockroaches twenty-four hours, fed them lumps of fat, and killed them after varying lengths of time. Large amounts of fat were found in the epithelial cells, and progressively less with longer and longer periods afterward. The periods of time used are not given. In some cases the cells were found so full of fat that they appeared as solid black, excluding all other structures from view. The fat found in the cells had evidently got there from the lumen of the crop, seemingly through pores. It was really a clear case of absorption by diffusion. In late stages of digestion fat globules were found lying free in the pouches of the body cavity behind the epithelial folds.

On the basis of Petrunkevitch's work, Sinety attacked the problem. He fed with fat fifteen hours and found fat beginning to appear in the cells. This is said not to be of special significance, but to represent fat which is absorbed from the blood by the cells and stored in them as reserve food material. His belief is that these reserves explain Petrunkevitch's figures.

Schlüter also based his work on that of Petrunkevitch, but used a special feeding method, namely, smearing of fat on the body and reliance on the cleaning activities of the animals to get the food into the mouth. He found that fat appeared in the cells and also in the blood lacunae fifteen to twenty minutes after feeding began. He assumed that most of this must have gotten through the intima and cells unchanged, for the splitting of fat to soluble products and their absorption could hardly have occurred so soon. The appearance was only seen in those parts of the crop where fat actually lay against the cells. A complicating factor was some residual fat, which was present in the cells when the experiment began. He never found cells completely filled with fat, but did find places where fat had rushed in from the lumen through a hole in the intima and had filled cells. Thus he explains Petrunkevitch's appearances of completely filled cells. In other experiments he starved animals eight days and fed them fat one to three days. None was subsequently found in the cells, but it was found there after eight days of sugar feeding. This led to his assumption that fat is made from carbohydrate in the body and is sometimes stored as fat in the cells.

Jordan in his text-book on digestion in invertebrates admits that much carbohydrate may be absorbed in the crop, but is doubtful as to fat, for the work of Sinety and Schlüter indicates that fat in the cells may be a product of metabolism.

In my experiments I used animals which had been starved two weeks to be sure no residue of fat was in the cells. As a matter of fact, no intracellular fat could be found after six days' starvation. The animals were fed a paste of olive oil and powdered sugar, and were dissected and fixed in Flemming's fluid after varying lengths of time, prepared by the paraffin method, and stained with acid fuchsin, In some cases Flemming's fluid was injected into the body cavity, but as this method gave no better results it was discontinued. In one series of experiments in fat breeding the following conditions were found in the cells of the crop: eight hours after feeding, small amounts of fat in the cells; after sixteen hours, small amounts; after twenty-four hours, moderate amounts; after thirty-two hours, large amounts; after forty-eight hours, maximum amounts, with many large globules of fat in the cells and in some cases the cells entirely black with fat to exclusion of all cell parts; after seventy hours, large amounts, the globules large and often seemingly in process of dissolution (figs. 3 and 4). At later stages more or less fat is present in the lacunae behind the epithelium. Controls were tried with a paste of sugar and mineral oil, the latter oil being indigestible, but without getting pictures of fat globules in the cells.

The following experiment also has an interest in this connection. A cockroach was fed a large amount of a paste of olive oil, sugar, and blue litmus powder, then its body cavity was entered and its crop ligated posteriorly. After forty-eight hours the animal was killed and the alimentary canal preserved. The crop showed a red content anterior to the ligation and this part was preserved in Flemming. Histological examination of transverse sections revealed many globules of fat in most of the cells. This fat had been absorbed here, and prepared for absorption by the crop secretion, for the stomach secretion could not have gotten here past the ligation.

To test whether fatty acids really could be absorbed, oleic acid was fed in a paste with sugar. The animals were dissected at intervals and their crops preserved in Flemming's fluid. Black globules of osmicated fat were readily found in the cells as follows: after eighteen hours, small amounts; after thirty hours, moderate to considerable amounts; after thirty-two hours, large amounts (fig. 6). So oleic acid is readily absorbed into the epithelial cells. The absorption is more rapid than in the case of fats, as might be expected, for fats must be split before absorption, while with oleic acid this is unnecessary.

In order to discover the relation between fats and fatty acids during the digestion of fat, a paste of sugar and olive oil was used as food; into the paste a generous amount of Nile blue sulphate was stirred. After three days the animal was dissected and the crop sectioned in a freezing microtome. The sections showed blue globules of fat in the epithelial cells. These were surely fat, for the stain colors fat blue, fatty acids red. Within the lumen of the crop and sticking to the intima a red mass was found, this was fatty acid derived from the fat eaten. In another case oil was stained bright red with Sudan III and fed. Subsequent frozen sections showed many red globules in the epithelial cells.

I have already stated that in some cases a large meal of fat may not all be utilized until about two months later. In these cases there is a continual absorption of fat in the crop throughout the whole period, as I have demonstrated by making a series of preparations at various intervals through the period of utilization of a crop's content of fat. Figure 5 shows a stage two months after a large meal was eaten. Absorption is almost completed, though some recently absorbed globules are seen in the region of the cells nearest the lumen. The middle region of the cells is occupied by large globules. The material of the globules is apparently being utilized, for the region between them and the body cavity is occupied by many small globules. These represent fatty material in process of passage (as soluble products) from the large storage globules to the blood in the body cavity. The large globules must represent storage products, ready to be utilized when needed. We have already seen that the lumen of the crop is important for storage of food, and now we see that the epithelium of the crop is important for storage, too, but in another sense.

A general survey of the facts I have described about absorption leads to the conclusion that the process of digestion of fat in the crop follows the usual course: fat is acted upon in the lumen of the crop by a lipase which in all probability comes from the cells as a secretion. This splits it to fatty acid and glycerol in the ordinary way. The soluble products are absorbed and resynthesized into fat by the cytoplasm of the epithelial cells and used by the body for its nourishment. As a first step in utilization, the fat passes from the cells to the blood plasma in the spaces behind the epithelium, and from here is carried to all parts of the body by the blood.

There is absolutely no doubt that the presence of fat globules in the epithelium indicates that the cells have absorbed fatty material from the lumen. The globules are practically always present at appropriate intervals after fat is eaten, and appear uniformly throughout great regions of epithelium, giving the same pictures through whole sections and whole series of sections in dozens of preparations. The globules may be seen in flat preparations and in transverse sections equally well, and may be demonstrated by staining with osmic acid, Sudan III, and Nile blue. Schlüter would have us believe that any fat in the cells had got there from the lumen directly through slits in the intima. This might occur occasionally; but an examination of my figures and my several lines of proof as given above, together with the great uniformity of my preparations and their agreement with those of Petrunkevitch, makes it absolutely certain that the cells of the crop absorb fatty matter in large amounts, and exhibit more and more of it in form of globules as digestion progresses.

When a comparatively small amount of fat is eaten, it does not fill the lumen, but leaves part of the epithelium untouched. This untouched part shows no absorption stages, while the cells on which fat lies show fat within. Schlüter found the same condition. This fact is additional evidence of the absorptive power of the crop; if the fat reached the crop by way of stomach and body cavity (as several authors contend it always does) it would surely appear fairly equally in all parts of the crop wall, and not merely in those regions which contain fat within.

Schlüter has emphasized that sugar may be synthesized to fat in the insect body. Such a process can in no way effect the explanation I have given of fat globules in the cells, even though sugar was always present in the foods I fed. We have seen that a mixture of sugar and mineral oil used as food gave no subsequent fat in the cells; if sugar could be readily synthesized to fat in these cells, the paste with sugar and mineral oil should give the same pictures as the paste with olive oil. This is not the case. We may conclude that all intracellular fat is derived from the fat of the food.

I have obtained indirect evidence that sugar is absorbed in the crop. Animals which had eaten a large amount of a thick paste of sugar and oil were dissected after two days. The food matter found in the crop consisted of clear, liquid fat only. The sugar of the food had gone, probably through absorption by the cells of the crop. The experiment suggests that the absorption of sugar occurs rapidly and in large amounts.

Gizzard. The large teeth of the anterior gizzard would suggest a grinding function, especially since they are surrounded by a thick layer of circular muscles. Plateau was the first investigator of the significance of the gizzard; he came to the conclusion that it served as a filter with a narrow lumen, the various chitinous projections, bristles, spines, etc., serving to hinder the passage of food. He admitted no trituration, for he found full-sized fragments of plant food and uninjured starch grains in the stomach after passing through the gizzard. Jordan and Ramme both state that the teeth move, not for masticatory processes, but for the mixing of food with enzymes and for crushing food particles to only a slight degree. The mixing in of the enzymes quickens digestive processes by aiding penetration. Mingazzini reached his conclusions from general biological considerations, and states that the complicated gizzard of Orthoptera cannot be a mere filter. This view is expressed by Biedermann in Winterstein's Handbuch. It is partly based on the assumption that such rapid eaters as cockroaches must needs have some chewing device. However, I have already described the retention of food from passing the gizzard for several days after a rapidly eaten meal.

In my experiments I have never fed food consisting of large particles, so of course I have never observed any appearances of mastication. Food is almost never found between the points of the teeth. I have found considerable pieces of chitin in the lumen of the stomach; some small ones are shown in figure 19. These must represent relics of food which passed through the gizzard without being crushed much. I therefore agree with the majority of authors on the subject in not emphasizing the chewing action of the teeth. As the contour of the teeth may be such that they fit very compactly together, this part of the gizzard may act as a sphincter, though not as a perfectly tight one. Figure 8 shows the spaces which are usually present between the teeth. It is not here that the main sphincter action of the gizzard resides.

Food is found between the teeth and also in the regions of the secondary and tertiary teeth and other projections. The food is evidently guided from crop to gizzard by following in the narrow channels between the projections. The teeth must open somewhat to let the larger particles pass. The movement is peristalsis of the crop, which is often very powerful when observed in the living animal.

As mentioned in the section on the crop, the gizzard is of importance in conducting forward the secretion from the stomach. For I have found an acid secretion in the hind crop behind ligations, and this seems to have flowed thence from the stomach. How the passage forward through the gizzard occurs has not been determined, but it seems probable that it flows in the narrow channels between the projections. These channels are the ones used later by the food in its passage backward. I have endeavored to demonstrate the forward passage of stomach secretion by injecting colored fluids into the stomach of the living animal. Such operations have always proved rapidly fatal.

Ramme studied the flow of the secretion in various Orthoptera, but not in the American cockroach. In some he found special passages which led from the origin of the coeca directly into the rear gizzard. I have studied serial sections of this region in the species I used, and have uniformly found no evidence of such passages. It may be that in some Orthoptera the coeca are special organs of secretion, so that a more direct passage would be advantageous. In the cockroach the coeca show the same secretory power as the stomach; a direct passage for their secretion would, therefore, have no significance.

The cushions of Miall and Denny deserve special attention. They are six in number and situated directly behind the primary teeth (figs. 7 and 10). Their surface is provided with needle-like spines, and these are especially significant on the surfaces directly toward the lumen. A definite band of striated muscle runs from this surface outward and upward to be firmly attached to the thick chitinous intima of the anterior surface of the teeth (fig. 7). Wilde has described the cushions as normally closed, their opposed surfaces fitting to form a tight joint. He considered that the tight closure of the cushions prevented the passage of food until it is well ground by the teeth. Basch thought that the cushions not only pushed against each other, but also pushed up closely under the teeth. Ramme has also described a close joint by the pressing together of the cushions by action of the surrounding ring muscles. Petrunkevitch has described the needles of the exposed surfaces as being attached inwardly to muscle fibrils, one fibril running to each needle. He believed that the contraction of the muscles caused movement of the needles by virtue of the attachment of the muscle fibrils to the bases of the needles. Ramme has ridiculed this view; he finds the muscles attached to the intima, not to the spines.

I have made many series of transverse and longitudinal sections of the gizzard, and never have I found the cushions closely appressed, and only in one case were they near each other. The cushions appear well separated from each other, as I have figured in transverse and longitudinal sections (figs. 7, 10, 15). As my preparations represented every stage of feeding and starvation, we may safely conclude that in the American cockroach the close opposition of the cushions occurs rarely or never and has no significance in the retention of food. The cavity of this region is practically always open for the passage of food. The appearances are much clearer if studied in longitudinal sections; such sections were evidently not used by most investigators. For the study of the intimate structure of the cushions great care in preservation and the changing of reagents is necessary. Flemming's fluid often causes a loosening of the intima and the destruction of its structure. Any preserving fluid may do this, or insufficient dehydration, or too rapid passage into paraffin. Only after many trials was I able to demonstrate the fibrillar structures leading to the needles as Petrunkevitch has described them. I believe that the other investigators took insufficient precautions with the material, thus destroying the intimate structure. Flemming's fluid is satisfactory if the material is left in it two days; Perenyi's fluid may also be used. It is necessary to use iron haematoxylin as a stain, followed by picro-fuchsin. Even then many preparations do not show the desired structure, for the fibrils and their connections are to be seen only in a limited area, and it is only by chance that sections can be made in just the right plane. In well-preserved preparations it is clearly seen that the muscles of the muscle band divide to fibrils (fig. 11) which pass between the epithelial cells and become little tendons. The tendons traverse the modified intima to attach to the socketed bases of the needles.

The muscle bands are six in number, and run from the intima of the cushions outward and upward to their origin in the thick and comparatively immovable chitin of the anterior surface of the teeth. The muscles are long and well striated; their contraction can cause little movement at their origins, so it must result in movements at the insertions in the cushions. The insertions are in the bases of the needle, and the sockets seem to allow them to move in response to muscular pull. Thus the structure certainly indicates that the muscles move the needles. A cross-sectional view of the muscle tendons which traverse the intima may be seen in sections cut nearly parallel to the surface of the intima, and just below its surface. Figure 12 shows such a picture: the tendons are seen within the matrix of the intima in the region between the epithelial cells and the surface of the intima. Such appearances disprove beyond question Ramme's contention that the muscles terminate in the inner surface of the intima. The fibers run not only to the intima, but through it (as tendons) to its outer surface and to the needle bases (fig. 11).

The needles are quite considerable in size, being 0.10 mm. to 0.15 mm. in length. Ramme has said that their extreme minuteness in comparison to the muscle structures precludes any mutual action, yet an examination of my figures reveals that the needles are much larger than the muscle fibrillae and tendons, and so connected with them that their movement by the tendons seems probable. The purpose of this movement is difficult to explain, but their position and arrangement suggest that probably they aid in moving food along through the gizzard toward the stomach, as Petrunkevitch suggested.

The region between the cushions and the narrow stomach passage is occupied by many folds of various sizes (figs. 7, 15, 16). These are all provided with spines, many of them being so inserted that they can lie flat on the surface. Several of the folds usually lie closely together, blocking entirely or partially the passage into the stomach. This arrangement enables them to hold back food, and I believe that in these folds the sphincter action of the gizzard is largely to be found. It is certain that the gizzard does act as a very efficient sphincter, holding back fluids for three days. We have seen that neither the teeth nor the cushions make a tight joint, so the only possible position for efficient retention of food is this rear part of the gizzard. Moreover, this is a logical position for a sphincter, for it is only necessary to protect the small opening into the stomach, the narrowest section of the whole alimentary canal; while in the more anterior gizzard, a much broader cavity would necessarily be closed by any structure acting as a sphincter. The arrangement of the folds is shown in cross section in figure 16, and in longitudinal section in figure 15. In both cases it is seen that the folds are surrounded by a considerable muscle sphincter, which brings about the appression of the folds by its contraction. The sphincter may be thicker than that which surrounds the cushions. Its position seems to indicate that it is the effective agent in making the gizzard the strong sphincter organ which it is.

Jordan states that in some insects the posterior, projecting part of the gizzard may project completely through the stomach when it discharges food, so that the food enters the small intestine and not the stomach. This adaptation results in protecting the stomach cells, which have no chitinous wall like other parts of the alimentary canal, from actual contact with the food. In the American cockroach I find that these conditions do not hold. Two facts bear out this conclusion: 1) longitudinal sections of gizzard and stomach show that the gizzard projects only a short distance into the stomach; even if the epithelial folds were flattened out it would fall far short of extending through the stomach; 2) when food which can be recognized is fed, it can be later found and identified in the stomach cavity. Therefore, it is certain that the function of the projecting gizzard is not to protect the stomach.

Ramme's idea of the function of the projecting gizzard is quite the opposite of Jordan's. He worked on cockroaches and other insects and decided that the projecting part does not serve to protect the stomach epithelium, but to direct food into a region of the stomach well below the coeca, these being diverticula of the extreme anterior end of the stomach. He asserts that the coeca are secretory in function, and that the hinder gizzard by directing the food into a region well behind them prevents their being clogged with food and so hindered in their secretory processes. I shall show that the coeca are not special secreting organs. Moreover, the fat I fed did enter the coeca in large amount. So the projecting gizzard does not serve to protect the coeca in Ramme's sense. It seems likely that the arrangement of parts prevents the hard and sharp pieces of food from passing into the comparatively delicate coeca.

Stomach and coeca. Various investigators have discussed the question of secretion in the insect stomach, and especially in that of the cockroach. The first phase of the question concerns the reaction of the secretion which is formed. Perhaps the first investigators in this field were Zuerst and Basch in 1858, who found the secretion in the anterior stomach neutral and in the posterior stomach weakly acid. Bellesme said that the secretion found in the stomach is acid and is derived from the coeca. Plateau in 1874 found the secretion slightly acid, but believed this to be accessory to the actual digestion, which he compared with pancreatic digestion. He removed the coeca and opened them on blue litmus powder. The powder was not affected, so he stated that the coecal secretion is alkaline. Krukenberg found the stomach secretion alkaline, while the acid secretion of the coeca flows into the crop to act on food there. More modern authors have uniformly found that the secretion of the stomach and coeca is acid in reaction.

In my investigations I first used the method of feeding a paste of sugar and litmus powder and dissecting the animals after various intervals. The contents of the coeca and anterior two-thirds of the stomach are always distinctly acid, while the contents of the hinder stomach may sometimes be alkaline. I have already given my reasons for believing that the acid secretion of the anterior stomach has its action not only in the stomach, but flows forward into the crop to aid in digestion there. This secretion is partly derived from the coeca and is no doubt very active in digesting all kinds of food, for the stomach is an important organ in secretion, digestion, and preparation of the food for the uses of the body. Schroder has proved that the coecal secretion has a digestive action on carbohydrates.

To make the matter of the reaction of the stomach more definite I removed the stomachs and coeca from ten animals, washed them in normal salt solution, and pressed out their contents. The ten stomachs and their coeca were ground with 10 cc. of salt solution and left for two hours. The mixture was then filtered and the filtrate titrated. The 10 cc. of the extract titrated against 0.3 cc. of 1/20 normal NaOH, thus indicating a distinct acidity. This shows that the digestive processes of the stomach occur in an acid medium.

The secretion, as Deegener and Jordan describe it, appears in little sacs which are formed at the ends of the cells and free themselves into the cavity. Holtz describes the pouring out of granules of secretion in Nematus. Steudel injected Congo red solution into the body cavity, and later found it appearing in more concentrated form in the extruded secretion sacs. Schroder holds that the sacs or lobes are not actual secretion, but that the secretion comes from a broader region of the cell. The projections may be for the secretion's dispersal, he says, and may form a broader surface for it.

VanGehuchten and Deegener have figured the release of spheres of secretion, and describe a cycle through which the secreting cells pass in three stages: 1) the formation of the sphere and its granular contents; 2) the completion of the sphere and its release; 3) the cell rests, meanwhile resembling a typical epithelial cell and probably being adapted to absorb soluble food substances. The process is described as continually going on, so that secretion is always ready for food when it arrives. The cells cannot form secretion spheres indefinitely long, but finally weaken and are cast loose with their last secretion sac. Several other authors have described the discharge of the oldest cells.

The appearances on my slides agree mostly with the observations of Deegener. The sacs at the ends of the cells and with their granular content are common, and many discharged sacs are seen in the lumen. Many regions of the epithelium show no signs of sac formation; these are in the resting stage. A density of the protoplasm near the ends of many cells indicates evidently a very early stage in the formation of a secretion of a secretion sac. All cells are able to secrete, provided they reach the surface, and the coecal cells show exactly similar sacs to those of the stomach, as we should expect from the similar histological structure. Both absorptive and non-absorptive cells are able to secrete. The absorbing cells are more active in secretion, perhaps because they are more mature. Every cell of a group of actively absorbing cells will very often show a large secretion sac. The presence of secretion is absolutely necessary before fat can be digested and absorbed; in several cases I found the lumen full of fatty substance, but no secretion sacs in evidence, and therefore no absorption. This must mean that cells of a large region of epithelium undergo a resting stage at one time; the stomach may thus be actively secreting and absorbing while the coeca do neither, even if stuffed with food. Or the coeca may be active while the stomach is not. These observations give evidence that the stomach and coeca are similar in function, and that the coeca may be considered as expansions of the stomach which offer a large surface for digestive processes. It also seems clear that the enzyme which acts on fats in the stomach is entirely derived from the secretion globules of the cells.

Jordan and others have found a fat-splitting enzyme in the stomach. Plateau found an emulsifying power, and Jousset showed the presence of an acid after the enzyme's action. I have verified that the enzyme is a true lipase, which splits fats to fatty acids and glycerol. I will not here describe in detail my method of determining the lipolytic action of the stomach, for I used the same method as in the experiments on the crop. The stomachs and coeca of thirty cockroaches were extracted in 30 cc. of normal salt solution. Of the 30 cc. of the extract 10 cc. were left in a flask at room temperature for seventy-two hours; to another portion of 10 cc. was added 10 cc. of olive oil. The mixture was shaken and treated like the first. Both were titrated against 1/20 normal NaOH. The portion without the olive oil titrated against 1.0 cc. of the alkali, while the portion with the olive oil titrated against 7.2 cc. of the alkali. This showed that the equivalent acidity of 6.2 cc. of alkali had been produced from the olive oil, and left no doubt that a lipolytic action had occurred and had produced the extra acidity through the formation of fatty acid as a digestion product of the olive oil.

The above results were obtained by using the stomachs and coeca together, so they did not make clear what separate parts the components took in the process. To determine this I dissected ten animals, removed the stomachs and coeca separately, put them in separate dishes, washed in normal salt solution, and pressed out the contents. To the ten stomachs was added 10 cc. of normal salt solution and the same to the eighty coeca. They were ground to a pulp in separate mortars, left two hours for the extraction of the enzyme, then to each was added the same amount of olive oil, and they were left seventy-two hours and titrated. The dish containing the coeca showed more acidity than the stomachs in the ratio 7: 5.

As the histological structures of the stomach and coeca are the same, a truer comparison of their lipolytic functions would be expressed in terms of equal areas of their epithelial surfaces. Examinations of freshly dissected and of sectioned material reveals that the coeca have on the average half the length of the stomach, and a third of its diameter. So each coecum has one-sixth the surface of the stomach, and the eight coeca have eight-sixths the area of the stomach. Thus the ratio of the epithelial areas of coeca and stomach is 8 : 6, and we have seen that the corresponding ratios of the lipolytic effects is 7 : 5. This close approximation is striking and makes it evident that the fat-splitting action of the epithelium of the stomach and coeca per unit of area (or volume) is the same. The coeca are therefore not the special organs of secretion which various authors have considered them.

The stomach and coeca are surely important organs for the secretion of digestive juices, at least as regards lipolytic action. Most authors have placed them first as secreting organs. They really occupy an important second place, the crop occupying first place and being about twice as potent in digestion, at least of fats, as the stomach and coeca combined.

Absorption in the stomach has been demonstrated by all investigators of insect digestion. All agree that the cells of the stomach have the power of absorbing food in liquid form. The older writers endeavored to demonstrate the passage of fat globules as such from the lumen into the cytoplasm of the cells, but the fallacy of this is evident since it is known that fats are split to soluble products before absorption. The descriptions of the process vary greatly as given by various investigators.

Among the earliest is that of Miall and Denny, who state in their text-book that the digestive products pass from the lumen through the protoplasm of the cells to the body cavity. We now know that the protoplasm has a selective absorptive action.

Petrunkevitch's opinion is, in brief, that in the stomach only the oldest cells, between the regenerating places, can absorb. Thus only a part of the inner surface of the stomach epithelium is capable of absorption. Digestion is considered as not very powerful here, but occurring mostly in the crop. The coeca are described as being structured similarly to the stomach and capable of absorption, but in less amount, for their function is mostly secretory.

VanGehuchten has described in one form a ring of special absorbing cells. This probably is not present in most insects.

Holtz has found that in Nematus the cells send out pseudopodia around the food and absorb it gradually. The pseudopodia are said to be distinct histologically and physiologically from the globules of secretion which are formed at the ends of the cells.

Cuénot found fat absorption in the posterior two-thirds of the stomach and in moderate amounts. The coeca are said to have a strong absorptive power. He thought the midgut was the only part of the alimentary canal capable of absorption.

Steudel in his work on cockroaches showed that only the non-secreting cells of the stomach were capable of absorption, and not the cells which were undergoing a process of formation of secretion globules. The unspecialized or resting stage is considered as an absorptive phase, which alternates in the cell with a secretory stage. He found conspicuous absorption of fat in the stomach and some in the coeca.

A great discussion has been waged over the question whether the stomach is the only organ capable of absorption. The absence here of the chitinous wall present in all other parts of the alimentary canal has led many on a priori grounds to believe that here and here only is absorption possible. Cuénot in 1896 stated that the stomach alone had the power of absorption-while the chitin of all other parts was impenetrable. Kowal, ewsky and Metalnikoff have used the method of pigment feeding, and conclude that almost all of the absorption occurs in the stomach. Metalnikoff fed food containing an iron salt; while it was being absorbed he precipitated the iron as Prussian blue; he found absorption in the stomach only.

Plateau, Petrunkevitch, and others, after thorough investigation, find the crop and important organ of digestion and the stomach of less significance. I have verified this, and further credence is given to this view by Sayce and others demonstrating that osmosis can occur through a chitinous intima.

My own investigations support those of Petrunkevitch. Fat is absorbed by the stomach cells and may be demonstrated in them by staining the tiny globules black by osmic acid. These globules first appear at very varying times after food is eaten, but no doubt only a comparatively short time after the food actually enters the stomach, provided secretion globules are present. Experiments with colored food have shown that fat may pass from the crop very soon into the stomach or may be entirely held back two or three days, even in animals which have been previously starved a long time. If the stomach is alone capable of absorption, as so many authors believe, it would indeed be hardly believable that an almost starved cockroach should be denied the power of absorbing or utilizing its food until several days after it has devoured it. What really happens is that the food begins to be absorbed and utilized by the crop soon after it is eaten.

The cells of the stomach are differentiated into older cells and regenerating ones; only the older cells can absorb. These usually occupy only a limited part of the surface of the epithelium, perhaps a third or less. The contrast between the absorbing cells and the others is striking, as shown in figure 21, where fat is being actively absorbed. Occasionally the upper ends of the neighboring cells may absorb a little fat. As this is only seen in the ends of the cells toward the lumen, it seems that fat is soon drawn from the neighboring cells through the lateral cell walls into the true absorbing cells. The cells which absorb most strongly secrete most strongly, as a rule, and there is almost no absorption unless secretion globules from some cells are present to act on the fat. Rarely the epithelium is not specialized, but shows all cells alike. In this case fat globules may be found in the ends of all cells but even here there is a tendency for certain groups of cells to absorb most of the fat. I cannot support Cuénot in his statement that only the posterior two-thirds can absorb, for I find fat in the cells of the extreme anterior stomach. Serial sections of the whole length of the stomach show similar cellular structure throughout the entire length and similar absorptive power.

The coeca give the same pictures as the stomach. Figure 20 shows the arrangement in alternate groups of fat-absorbing cells and regenerating cells. I conclude that the coeca have approximately the same absorptive power per unit of surface as the stomach does. The fact that the coeca both secrete and absorb in just the same way as the stomach indicates that they are to be considered as proliferations from the stomach merely for the sake of exposing more stomach epithelium to food.

It does not seem to me at all probable that the limited amount of fat absorption observed in the stomach can be all the body is capable of. Only a part of the epithelium can absorb, and, moreover, more than half of my preparations of stomachs with fat in their lumina showed little or no absorption in the cells. This was due to lack of secretion or some other deterrent factor, and goes to show how irregular and comparatively slight is the absorption of fat in the stomach as compared with absorption in the crop, where I was practically always able to find good absorption stages at appropriate intervals after feeding.

The stomach of Dytiscus gives very similar pictures to that of the cockroach, though the coeca are small and numerous. A specimen was fed suet and dissected after three days. The stomach epithelium showed absorption of fat in groups of absorptive cells separated by regenerating cells, just as in the cockroach. The coeca also showed the same structure and absorptive power as the stomach.

In connection with the question of digestion in insects, a striking phenomenon has been described as occurring in the tracheae of the alimentary canal. This is the question of the presence of fats and other substances in the tracheal tubes after feeding, which has been under discussion more or less for about seventy years.

In 1847 Alessandrini noticed indigo in the tracheal tubes of moth larvae after they had eaten food containing indigo, both in the tracheal and cells and in the lumen of the tubes. Stimulated by this finding, Bassi did further work on the same material and concluded that Alessandrini's work was correct, but that only certain animals, and often only certain parts of these, showed the phenomenon, continuing to show it not only through the larval, but also the pupal and adult stages.

Blanchard injected pigments into the body cavity and found them later lying along the inner surfaces of the tracheal walls. He believed that they were carried there by the blood stream and named the process 'peritracheal circulation.' His results were quite uniform, and he considered the variability of Bassi's results to be due to variation in the amount of stained food previously eaten.

Soon after this Agassiz started his investigations on the problem and arrived at the conclusion that there are two sorts of tracheae, one respiratory and one circulatory, the two differing considerably histologically. These findings have not been substantiated by other authors.

Sadones found that there are, however, two distinct kinds of tracheal endings, one in the epithelium and one in the tissue below. Petrunkevitch has verified this.

Faussek described in certain insects tracheal tubes which enter the epithelium of the stomach and possess a distinct lumen bounded by plasma and normal nuclei, but no chitinous boundary. These were stated to end in sacs bounded by one or two nuclei. The appearances of sacs have since been criticized, and I agree with the critics that the sacs were probably artifacts caused by diffusion or currents. Free ending of tracheae were also described. This is in disagreement with the ideas of most investigators except Vieweger. The latter has described free intracellular endings of tracheae in the cells of various tissues; these tracheae are said to branch in the plasma of the cells, some of the brachlets passing into adjacent cells. I have never been able to find any such striking appearances in my preparations.

Petrunkevitch described the end cells of the tracheae, and figured the two types which occur; one type forms an integral part of the epithelium of the crop and resembles the other cells except for its narrowness and the tracheal connection below; the other type lies immediately below the ordinary epithelial cells and does not extend to the intima (fig. 2). Within each type of cell may be found the minute ending of the lumen of a tracheal branchlet. The tracheal tubes are bounded throughout by peritracheal cells, in which nuclei are always visible, but whose cell boundaries are not defined.

Petrunkevitch has also described four very interesting appearances which occurred in preparations made at intervals after fat had been fed. These four phenomena are: 1) the presence of many tiny globules of osmicated fat in those tracheal end cells which form an integral part of the epithelium, the globules being identical with those of the ordinary cells, and evidently representing fat absorbed from the lumen; 2) fat in the tracheal tubes, usually lying along the inner walls of the tubes and upon the coils of the spiral, supporting structures, and even moving along the spirals or moving out into the lumen to lie in a sticky deposit there; 3) the presence of globules of osmicated fat in the peritracheal cells; 4) the presence in the lumen of certain tubes of a sticky deposit which resembled the contents of the crop's lumen, and which sometimes contained leucocytes and fat globules. These four appearances were described as representing four stages in one process which occurred as follows: The at is absorbed by the tracheal end cells and passes from their cytoplasm into the minute terminal lumina of the tracheal tubes. In these the fat tends to lie on the spirals, and to proceed along the tubes in a spiral course by slipping along the spirals. It is now absorbed by the peritracheal cells and utilized by them. Not only fats are passed into the tracheal tubes through the tracheal end cells, but also other foods, and these appear as a sticky mass in the lumen. This mass was called chyme; it often contained cells which exactly resembled leucocytes. These cells seem to devour fat and other foods.

Schlüter employed methods somewhat similar to those of Petrunkevitch, but has disagreed with him in almost all the details described. He is unable to find any evidence of absorbed fat in the epithelial or tracheal end cells. I have carefully examined the data of his experiments which he has published, and I find that the time intervals he used were so chosen that only in a very few cases would absorption probably be in evidence. In only two of his experiments as given would significant absorption be expected, judging from my results. These two experiments apparently represented single animals, so it seems that the chance individual variation of a very few animals from the usual absorptive function really explains Schlüter's denial of absorption in the epithelial and tracheal end cells.

Schlüter assumed that appearances of fat and chyme in tracheal tubes indicated a chance pushing or slipping of substance from other regions into the tracheae during sectioning of the material or preparation of the slide. Or the contents of the tubes may have entered them by capillary action during dissection. Schlüter's final objection is that as such appearances have no reason nor physiological explanation, they have no actuality.

In my first experiments I found stages which corresponded very closely with those of Petrunkevitch. I used animals which had been starved two weeks and fed them a paste of olive oil and pulverized sugar. The animals were removed after certain intervals and dissected, immersed in normal salt solution. The crop was slit longitudinally so that the contained food might float out, the tracheal trunks of the crop were cut and the crop was removed and placed in Flemming's fluid. Flat mounts of the wall of the crop were made and also paraffin sections. Such preparations revealed the presence of black fat in the tracheal tubes, either in some of the smaller tracheae or in both large and small tracheae. The pictures corresponded so closely with those of Petrunkevitch that I believed I had verified his work, and I so stated in a preliminary paper.

In attempting to analyze the explanation and significance of the intratracheal fat, I soon found that if I bathed a cockroach in olive oil, the oil entered the spiracles readily and filled some of the tracheal tubes to a considerable extent. Schlüter has suggested, too, that some fat might enter the spiracles. This showed plainly that in work with tracheal tubes a new factor must be considered, namely, the sucking action of the tubes due to negative pressure. This factor was not used or eliminated in experiments by previous authors, and I now realized that I must always consider it in further experiments.

Moreover, the fact that olive oil enters the spiracles affords an entirely new method of attacking the problem. The oil enters during the bathing process because it is sucked in by the respiratory movements which cause negative pressure within; oil is sucked in until pressure is equalized throughout the tubes, so that all air which is contained in the tubes is now at atmospheric pressure. The animal may now be bathed in the oil until it dies through suffocation due to blocking of the spiracles and therefore its breathing movements cease. Complete equalization of pressures throughout the tracheal system is then assured, and consequently there can be no subsequent sucking action. Knowledge of this process allows a new method of investigating whether or not fat is normally present in the tracheae after stained fat has been eaten. For an animal can be bathed in oil which contains a stain of contrasted color to that of the stain which the food contained, this differently stained oil used for bathing will then enter the spiracles and equalize the pressures in the tubes, so all possible danger of sucking action in them during subsequent dissection is obviated. As red oil was used in feeding, green oil would be ideal for the bathing process. However, no brilliant green stain for fat was available, so I used a green solution which was found to enter the spiracles and tubes just as oil did. This was a mixture of equal parts of glycerin and 70 per cent alcohol, and saturated with brilliant aniline green. By using this mixture to bathe until they died animals which had previously eaten red fat, it is evident that the following set of conditions would be given: green fluid in the main trunks, preventing entrance of any other substance from without; air in the remainder of the tubes at normal pressure, so there is no danger of sucking action anywhere even if tubes are injured or cut; red, if any, in the tubes, indicating a normal content of red fat in the tracheae due to metabolic processes and by no chance to the conditions of the experiment. The actual set of experiments is the following:

One hundred and twenty-six cockroaches were fed a paste containing powdered sugar, olive oil, and Sudan III, these were removed at intervals. Eight hours after feeding three of them were removed from the jar, their necks were tightly ligated with silk to prevent any fluid used in the experiment from being swallowed, and they were immersed in the green mixture of glycerin and alcohol. After a short period of extreme activity, the animals died by suffocation, due to the liquid entering the breathing tubes; they were then removed from the green solution, washed, and dissected. It was found that the green had penetrated a considerable distance into the tracheal trunks, it had been drawn in by the sucking force present in the tubes before the animal died. The presence of this green fluid in the tracheae during the dissection prevented the entrance of any other substance into the tubes, not only by way of the spiracles, but at any place, for pressures were equalized. The trunks were cut in the region containing the green and the alimentary canal was carefully removed without injuring the wall of the crop. It was mounted in toto in glycerin and examined microscopically for the presence of any red substance in the tubes; such red substance would if present mean that red fat had been derived normally from the food in the crop's lumen. But no red fat could be detected in the tubes.

Three more of the animals were removed and treated similarly sixteen hours after feeding, three more at twenty-four hours, and three more at the end of eight eight-hour period throughout two weeks. In none of the 126 animal used could distinct redness be observed in the tracheae. Other animals were fed and dissected under the same conditions except for the fact that the crop was slit longitudinally during dissection; these showed no red substance in their tracheae, nor did a series of animals which were treated similarly but for the fact that their crops were preserved in Flemming and sectioned; microscopic examination of sections of this material revealed no osmicated fat in the tracheae.

The experiments had comprised all usual digestive pe iods and had included so many animals that individual variation and other complicating factors were negligible. The result was considered final and definite; it was certain that fat did not normally enter the tracheal tubes, as I had previously thought. I realized that all the fat I had seen in tracheae in earlier preparations must have come from an abnormal source.

I next attempted to analyze why my earlier experiments, like Petrunkevitch's, showed fat in the tubes, while more careful controls did not. From previous observations with colored fat I knew that oil might enter the cut ends of large tracheal trunks, but obviously this did not explain why Petrunkevitch and I had found fat present in only certain small branches and groups of branches. Another factor had obviously been acting. In attempting to explain this I performed the following experiment: An animal which had eaten some red paste two days before was pinned, while still active, dorsal side down on a dissecting pan. The spiracles were then thickly smeared with celloidin solution. This soon dried and formed a complete film over all the spiracles, thus preventing the entrance of any substance into the tracheal trunks within. The passage of air was prevented, but the still living animal performed to some extent breathing movements. The result of these movements is suction, but nothing could be sucked in, so the suction existed in the tubes without any possibility of anything being sucked in. The animal's body wall was carefully slit and pinned to the side without dirturbing the tracheae. Now the wall of the crop was slit longitudinally. This necessarily involved cutting many of the tracheal branches of various sizes which ramify over the crop. As soon as the animal was surely dead, the crop was removed and its outer surface examined microscopically. The small tracheae which had been unavoidably cut were especially observed, and it was found that some of them contained red fat in the region of the cut ends and for a distance beyond. This surely did not represent a normal process, but resulted from some factor which had been acting through the experiment. It seems certain that the negative pressure in the tracheae, as mentioned above, had exerted a sucking action at points where openings were made. Such openings were made when the crop was slit longitudinally, and as a result the substance at the cut end (in this case olive oil) had been drawn in. This experiment was repeated on other animals and showed conclusively that the fat which Petrunkevitch and I had found in minute tracheae in our preparations had been sucked in by the tubes through their cut ends during the dissection, and was therefore no normal content, but an artifact. We had easily overlooked it because such small structures were involved and because we had used colorless fats. It was interesting to explain why Petrunkevitch found fat in the tubes only at considerable intervals after feeding, while I found it at many stages. This is simply due to the fact that Petrunkevitch fed solid fat which required some time to be liquefied and changed to fatty acid and thus made capable of being sucked into tubes. In my work, liquid fat or fatty acid was always present in the crop after feeding, and it was thus always possible that it be drawn into the tracheal branches by suction, no matter at what period after feeding the cut was made.

The above experiments demonstrate that fat in tracheal tubes is abnormal; its presence may be accounted for in three ways: 1) Suction of fat through minute tracheae which are cut when the crop is opened in dissection; this suction may occur centrifugally or centripetally; 2) suction through large tracheal trunks and through spiracles; 3) capillarity. These three factors seem sufficient to explain the pictures of the several investigators who have so long discussed the problem; they indicate that fat is never normally present in the tracheal tubes.

The other three appearances which Petrunkevitch described and figured must now be explained, and, firstly, the globules of osmicated fat in the tracheal end cells. I have verified this in various preparations. These cells resemble in structure the epithelial cells, so it is natural that they absorb soluble food products in the same way. It cannot be definitely stated whether the fat gets merely into the end cells or also into the first of the peritracheal cells, for cell walls are indistinguishable. I do not believe that fat ever gets from the cytoplasm of the tracheal cells into the lumen of any tracheae.

The fat globules found in the peritracheal cells are easily explained. They only appear occasionally in my slides, for the cytoplasmic layer is very thin in the American cockroach (fig. 14), and not of such thickness as in the oriental cockroach. The peritracheal cells are bathed by the blood which fills the body cavity, and they take from the blood the food products which they need. The blood contains much fatty substance after fat has been eaten, and this substance may readily be absorbed by the peritracheal cells and reorganized to fat, which appears in globules in the cytoplasm. Similar globules may also be seen in the cells of the Malphigian tubules, which are similarly bathed by the blood.

The last phenomenon described by Petrunkevitch is the presence of chyme and leucocytes in the tubes. I have found such chyme present in practically all my preparations of the crop, and leucocytes were often present in it (fig. 14). It was present even in the most careful controls, where the animals were immersed in the green solution before dissection; it usually appeared in some of the smaller tracheae, but sometimes in the larger ones. There is no doubt that the presence of a sticky deposit is a common occurrence in some tracheae of American cockroaches. Its origin is very difficult to explain. In view of experiments already described, it is certain that it does not enter the tubes through the tracheal end cells. My explanation is only tentative and cannot be tested experimentally nor verified by observation. It is well known that many Orthoptera have difficulty in moulting, often dying or sustaining injuries in the process. It seems possible that during the moulting of cockroaches slight disturbances might cause rips or breaks in the tracheae. This would be fatal if it occurred much in the larger tracheae, but might cause little inconvenience if it happened in the smaller branches. Blood would then fill the torn branches with its contained leucocytes. The deposit in the tracheae is always much thicker than blood; it may be that some of the water diffuses out of the blood in the tubes. Perhaps the leucocytes ingest some of the unnatural substance present. The following experiment was performed to determine whether leucocytes might enter tracheal tubes which contained an unnatural substance.

A normal animal was immersed in olive oil which was stained red. The animal was dissected, and the tracheal trunks were found to contain much red fat. A piece of one, filled with fat, was removed and inserted into the body cavity of another normal animal. This animal was dissected three days later; the red trachea was removed, preserved in Flemming and sectioned. The tube was found to contain many leucocytes which had entered in response to the unnatural substance, and were ingesting it, as evidenced by the globules of ingested fat in their cytoplasm. The result of the experiment suggests that leucocytes may enter any rips which occur in the tracheae and may ingest any foreign material there, whether fat, thickened blood plasma or other substances. Such a theory is incapable of proof. It is almost certain in any case that the chyme is not to be considered as a really normal content.

My investigations have shown that three of Petrunkevitch's four striking appearances represent usual conditions, his slides agreeing closely with mine. Therefore Schlüter's absolute and critical denial of his observations is unjustified. As to the interpretation of these four observations, I am convinced that they represent four distinct processes, two of them normal, and not four stages in one process, as Petrunkevitch thought.

Blanchard's 'peritracheal circulation' theory should be considered here. In order to test whether substances really can pass from the blood into the tracheae, as Blanchard described, various substances were injected into the body cavity and later searched for in the tracheae. Such injections had little or no toxic effect. The best substance to use is phenol-sulphonephthalein, which passes through the body dissolved in the body fluids. The presence of this stain could not be detected in the tubes at any of several periods after injection, though the stain was absorbed by the stomach and intestine. Perhaps Blanchard's conclusions of 1849 may be due to poor definition of tracheal lumen and peritracheal cells on his slides. My experiments support Petrunkevitch in stating that there is no evident peritracheal circulation in the cockroach.

In conclusion, I wish to thank Professor Petrunkevitch, under whose direction the work was done. I wish, too, to thank Professor Mendel for his advice concerning some of the experiments, and Professors Osborne and Mendel for some of the reagents used; also Mr. W. S. Sturges for assistance with the titrations.

SUMMARY

1. The crop is the principal organ of digestion in the cockroach. Its enzyme splits fats to soluble products. The progress of digestion may be observed by giving food containing indicator stains. Digestions may be carried on outside of the body, and quantitative estimations of the amount of digestion may be made by titration. Such tests show that the crop is at least three times as active as the stomach in fat digestion.
2. The crop is the principal organ of absorption of fats. The process is rather slow and reaches a maximum two days after the fat is eaten. The absorption of one large meal may take two months. All cells of the epithelium of the crop can absorb; the cells may store globules of fat until it is needed.
3. The crop is an important organ for storage of food; it may store enough to supply the needs of the animal more than two months.
4. The gizzard has an important sphincter action, and may withhold food from the stomach several days. The sphincter action resides in the folds which protect the passage from the gizzard into the stomach.
5. The needles on the six significant folds or cushions of the posterior region of the gizzard are moved by special muscle fibrils and tendons derived from long strands of striated muscle. The function is difficult to ascertain, but possibly they aid in moving food through the gizzard.

6. The coecal epithelium has the same function and histological structure as that of the stomach. It exhibits somewhat more surface than the stomach, so that the digestive (enzymatic) power is somewhat greater than that of the stomach. The secretion appears as little spherical sacs released from the cells.

7. The stomach and coeca absorb food in groups of absorbing cells. These groups are separated by groups of undifferentiated and immature cells which will later take the place of the older ones as they are cast at intervals.

8. The tracheal end cells absorb fatty products from the lumen of the crop, and the peritrachèal cells absorb them from the blood. The tracheae themselves never contain fat normally. The presence of fat and other substances in the lumina is due to imperfect methods of preparation.

9. Certain tracheae of practically all animals contain a sticky substance whose origin is uncertain. Leucocytes are often present in this substance.

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PLATES

ABBREVIATIONS

AE, group of absorptive cells of stomach
AM, anterior muscle sphincter of gizzard
CA, group of absorbing cells of caecum containing absorbed fat
CC, cells of epithelium of cushion
CE, epithelium of caecum
CH, chitinous pieces in lumen of stomach
CU, cushion
DC, deposit in tracheal tube
EC, epithelium of crop
FC, muscle fibers of crop's wall
IC, intima of cushion
IE, intermediate cells of stomach epithelium
LC, leucocyte
MB, fibers of one of the six muscle bands
MF, fibrils derived from fibers of one of the six muscle bands
MT, tendons derived from muscle bands
NE, needles
PC, peritracheal cell
PM, posterior muscle sphincter of gizzard
PS, passage from gizzard to stomach
PT, primary teeth
RF, radial muscle of crop
ST, secondary teeth
TT, tertiary teeth
TC, tracheal end cell
YC, young and embryonic cells of stomach epithelium

PLATE 1

EXPLANATION OF FIGURES

1 Section of wall of oesophagus, showing the processes of its surface, the intima, the cells, and a few muscle fibers. Flemming. Acid fuchsin. 525 diam.

2 Transverse section through the wall of the crop, showing the cells and the intima, the ends of tracheae and the attachment of a radial muscle to the intima. Flemming. Acid fuchsin. 325 diam.

3 Transverse section through wall of the crop, showing the result of absorption of fatty material thirty-six hours after ingestion of olive oil. The cells contain many globules of osmicated fat. Flemming. Acid fuchsin. 325 diam.

4 A later stage of the same process. The cells contain a maximum amount of absorbed fat. Flemming. Acid fuchsin. 325 diam.

5 Transverse section of crop of animal which had eaten a large meal of olive oil two months previous to fixation; no other food was given. The cells contain globules of stored fat, which are being gradually broken down and utilized. Flemming. Acid fuchsin. 325 diam.

6 Section through wall of crop, showing the result of absorption of oleic acid thirty-two hours after ingestion. Flemming. Acid fuchsin. 325 diam.

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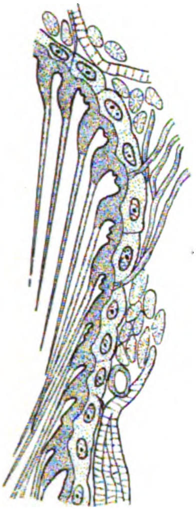

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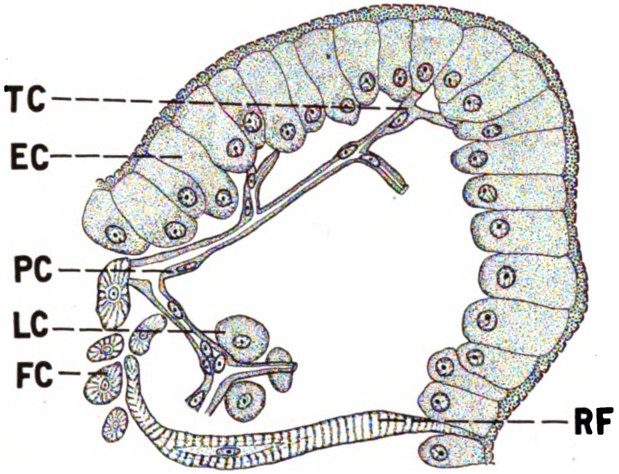

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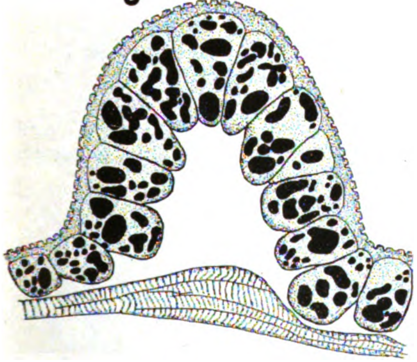

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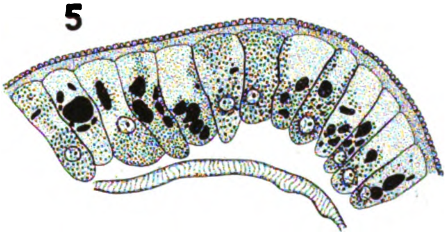

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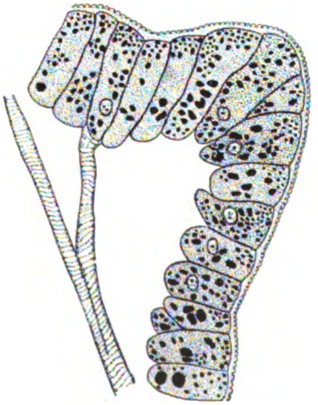

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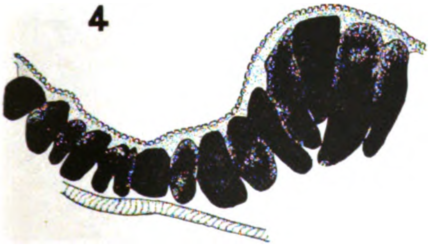

PLATE 2

EXPLANATION OF FIGURES

7 Longitudinal median section of gizzard, showing the heavy teeth, the cushions behind them, the folds of the posterior part, and the passage to the stomach; also the circular sphincter muscles of the anterior and posterior regions and two of the longitudinal muscle bands. Flemming. Iron haematoxylin and picro-fuchsin. 35 diam.

8 Transverse section of anterior gizzard, showing the teeth and the surrounding circular muscles, also the six longitudinal muscle bands in cross section. For details of the region between the teeth see figure 9. Flemming. Acid fuchsin. 35 diam.

9 Transverse section of the secondary and tertiary teeth of the anterior gizzard; showing also the epithelium and circular muscles. Flemming. Acid fuchsin. 90 diam.

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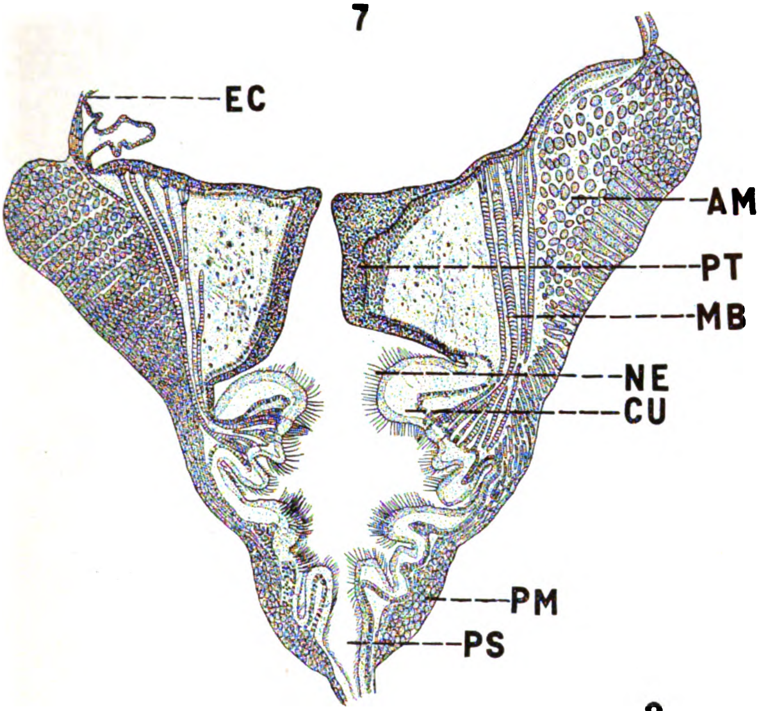

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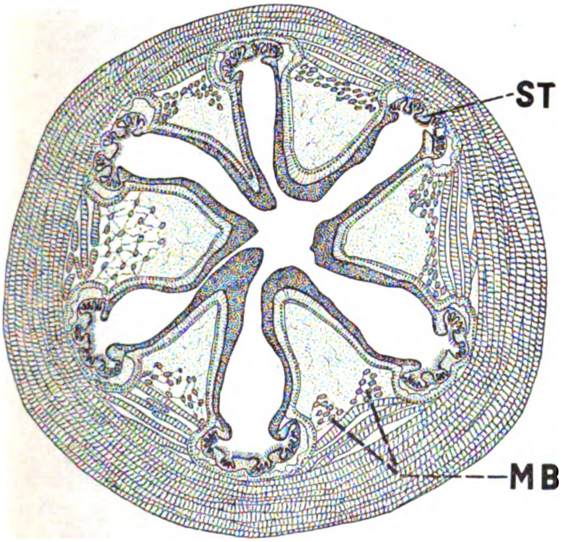

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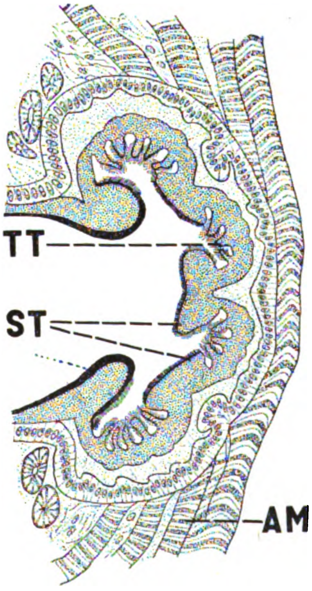

PLATE 3

EXPLANATION OF FIGURES

10 Transverse section of the gizzard in the region of the six cushions, showing the passage of the lower ends of the six muscle bands through the cushions to the needles on their surfaces. Flemming. Iron haematoxylin and picro-fuchsin. 35 diam.

11 Detail of the connection between the muscles and the needles of the cushions. It is shown that the striated fibers divide to fibrils, themselves striated. These pass through the epithelium and become tendinous. The tendons cross the matrix of the intima and insert in the bases of the needles. Flemming. Iron haematoxylin and picro-fuchsin. 200 diam.

12 Section through intima of a cushion, nearly parallel to surface, showing in transverse and diagonal section the tendons which traverse the intima. Zenker. Iron haematoxylin and picro-fuchsin. 90 diam.

13 Surface view of a group of bristles of the cushions, showing the chitinous projections to which they are movably attached. 200 diam.

14 Section through a tracheal tube which is filled with coagulated deposit, in which leucocytes may be seen. Flemming. Acid fuchsin. 350 diam.

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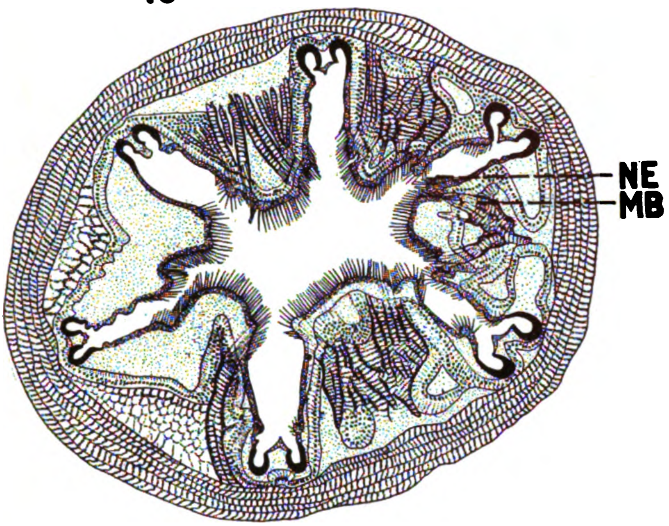

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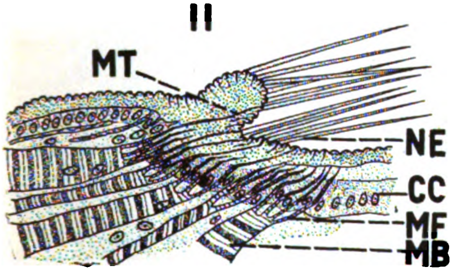

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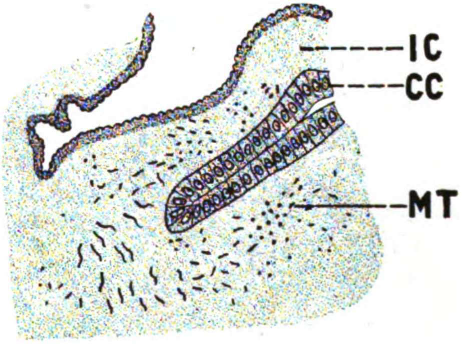

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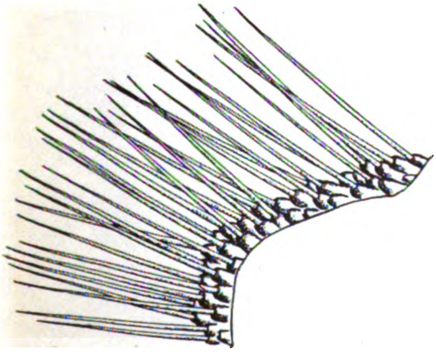

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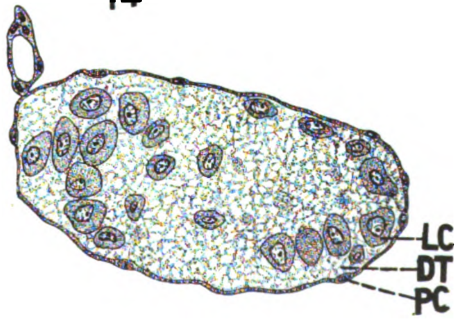

PLATE 4

EXPLANATION OF FIGURES

15 Longitudinal section through hinder end of the gizzard, showing the closely appressed folds of the inner wall and the thick surrounding sphincter muscle, which seems to make a tight closure. Flemming. Acid fuchsin. 35 diam.

16 Transverse section of the same region, showing the folds almost closing the passage, and the surrounding sphincter. Flemming. Acid fuchsin. 60 diam.

17 Transverse section of the wall of the crop, showing in one place an abnormal appearance or 'lesion' of the epithelium. Flemming. Acid fuchsin. 325 diam.

18 Surface view of epithelium of the crop, showing unusual conditions. Several binucleate cells are present, and also a region of cytoplasm where cell walls are lacking. Perenyi. Ehrlich's haematoxylin. 200 diam.

19 Transverse section of stomach two days after ingestion of fat. The epithelium shows frequent dark regions which indicate absorption of fat here. The lumen of the stomach contains oil drops, pieces of chitin, and other substances. Flemming. Acid fuchsin. 35 diam.

20 Transverse section of a caecum, prepared in the same way as the stomach of figure 19, and showing a similar condition of fat absorption. 35 diam.

21 Transverse section of epithelium of stomach's wall after ingestion of fat, showing in more detail the groups of cells which absorb fat, and also the embryonic cells which continually make new cells to replace the lost ones. Flemming. Acid fuchsin. 200 diam.

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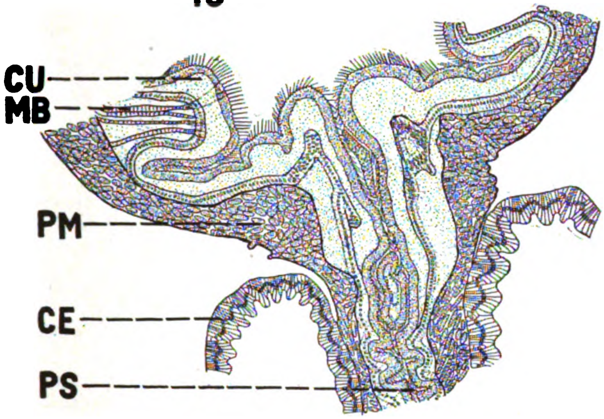

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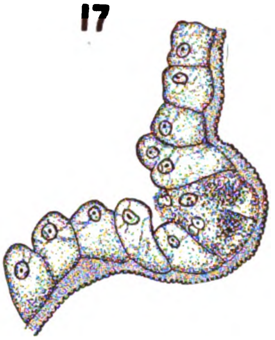

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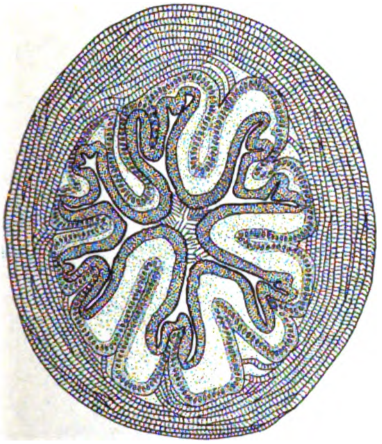

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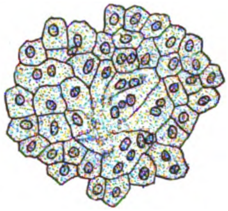

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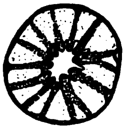

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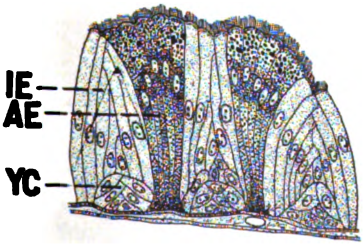

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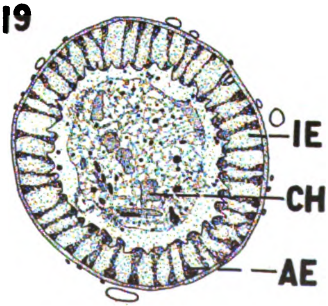

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CONCERNING A NEW METHOD FOR THE BIOLOGICAL STANDARDIZATION OF PITUITARY EXTRACT AND OTHER DRUGS

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PRELIMINARY

It is a fact familiar to all physiologists that a number of chemical complexes which are of great importance in medical practice have thus far defied chemical analysis. Drugs of this type must consequently be subjected to biological standardization. In the following pages we shall point out the disadvantages of certain standardization methods now in use and describe a new method for the assaying of pituitary extract which we believe to have advantages both in economy of material and time and in the uniformity of the standardized product. The application of the new method to drugs other than pituitary extract appears to be merely a question of technical detail.

PREVIOUS METHODS

In the two methods which are at present used for the assaying of pituitary extract the strength of the drug is tested by its action upon, (1) strips of virgin guinea-pig uterus or, (2) the normal blood pressure of dogs. Both methods involve two important groups of variables. (a) Considerable variation occurs in the strength of samples of pituitrin taken from different individuals of the same species. This appears to be due principally to the individual variations of the animals and also in part to variations in the details of the extraction processes. (b) Great variations occur among the test animals. Frequently the uterus is so sensitive that it becomes quite useless as a test object (Roth, 2). Again, the sensitivity of the blood pressure of dogs varies within wide limits. Such arbitrary standards as "healthy" or "carefully selected" are open to the objection of subjective interpretation. Furthermore, the same strip of uterus or the same dog is used repeatedly, which permits of no exact control experiment. According to Hamilton and Rowe (1) "The isolated uterus test, while in some respects logical, is not in its present form practicable to be used as a commercial method for the final standardizing of pituitary extract."

It is obvious that if tests could be carried out upon physiologically comparable units of tissue taken from the same animal the factor of individual variation would be automatically eliminated. In the new method which we describe in the following pages the reaction times of two physiological units—two groups of pigment cells—which have been physiologically tested and found to be identical, are compared in a standard and an unknown solution. Each piece of tissue is used for one test only and, since the pieces are taken from a single animal the chances of individual variation are eliminated.

THE NEW METHOD

The effects of a number of physiological and chemical stimuli upon the melanophores of F. heteroclitus have been studied in considerable detail during the past seven years (3). As a result of this work it appears that the melanophores of Fundulus and probably of all other teleost fishes must be considered functionally modified smooth muscle cells (4). This conclusion has opened a new approach to various pharmacological problems including that of biological drug standardization.

TEST ANIMALS

The "killiefish" or "mummiechog," F. heteroclitus, is a common minnow occurring in great numbers along the Atlantic coast. For experimental purposes it has the advantages of being easily obtained and of possessing an astonishing tenacity of life. Fish of a suitable size—7 to 10 cms.—may be caught with a dip net or minnow seine in any small tide water stream or salt marsh ditch from April to November in the latitude of New York.[1] A baited "umbrella net" may also be used to advantage. If the fish are brought into the laboratory in October they may be kept in good condition throughout the winter in large shallow tray tanks[2] about 5 by 8 feet, containing 2 inches of sand or gravel and 3 or 4 inches of water. If the temperature is low—about 5 to 12°C.—from 100 to 125 fish may be kept in a single tank. It is not necessary to keep the fish in sea water. They will live for weeks without apparent injury, in tap water, but indefinitely in sea-water diluted 15 to 30 times. The fish should be fed once a week on clams, earth worms or chopped raw meat scraps.

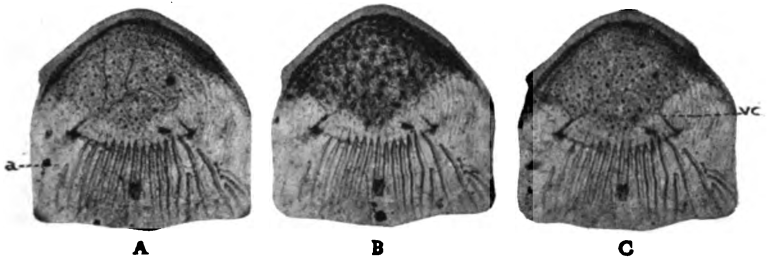

FIG. 1. SUCCESSIVE MICROPHOTOGRAPHS OF THE SAME SCALE
a, the melanophores contracted in the standard potassium chloride solution; *b*, the melanophores re-expanded in 0.1N NaCl and *c*, a second contraction of the melanophores in a solution of pituitary extract. A comparison of *a* and *c* shows that in the latter, following the treatment with pituitary extract, the peripheral blood vessels have contracted. The corpuscles have been forced into the wider portions of the vessels, *vc*. The exposed bony portions of the scales serve as a convenient handle for manipulating the scales.

THE TEST OBJECT

In a previous paper (3–'13), we described in detail the topographical arrangement of the melanophores in the skin of Fun-

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[1] During the winter months certain of the fish markets of Boston and New York have "killies" on sale for pickerel bait. The average cost is a cent apiece.

[2] The tanks are easily constructed of 1 inch planed white pine boards which should be fastened with screws. The tanks are rendered water tight by thoroughly ironing in soft paraffin with an electric iron. The paraffin will not injure the fish and the tanks may be used immediately.

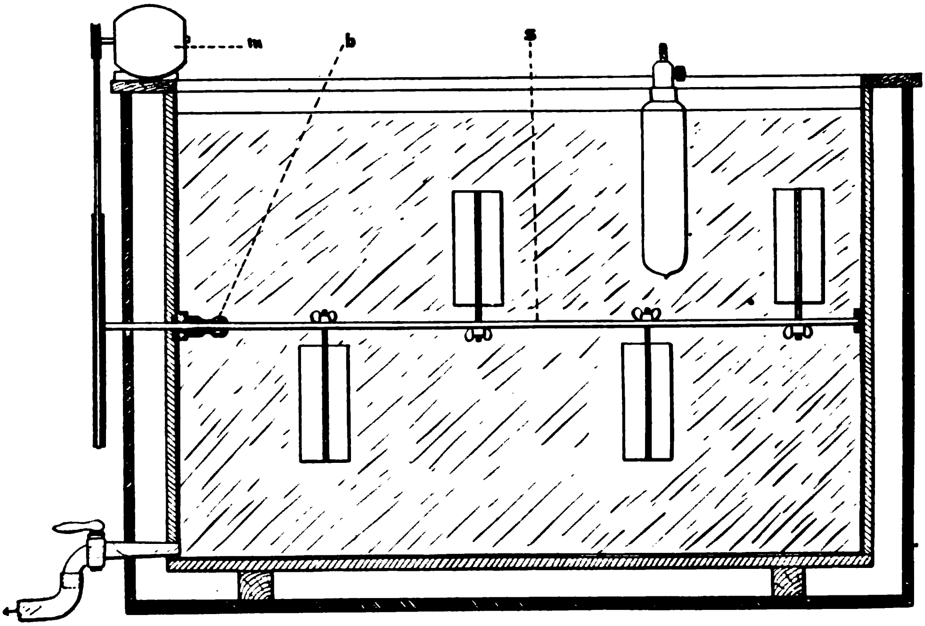

FIG. 2. SCHEMATIC MEDIAN SECTION THROUGH THE THERMOSTAT RESERVOIR

The thermoregulator and cooling device are not shown. The water is drawn off through the faucet at the left and passes to the warming stage. A siphon regulator keeps the water at a constant level. The space between the wooden walls is packed with asbestos wool.

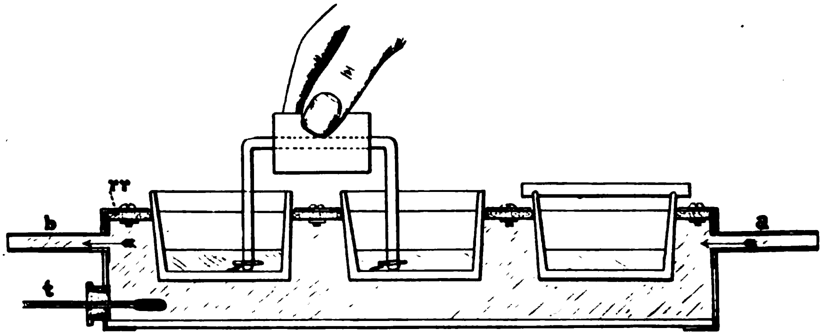

FIG. 3. THE WARMING STAGE

Showing a pair of scales just being immersed in separate solutions by means of the dipping device. The letters refer to the description in the text.

dulus. Each scale consists of a bony plate, a thin dermal layer containing the superficial melanophores and a thin, non-pigmented sheet of epidermis. The melanophores and epidermis cover about half the surface of the scale. The exposed bony portion of the other half (*a*, fig. 1A) serves as a convenient handle for transferring the scales from one solution to another. When a scale with widely expanded melanophores is placed in a pituitary solution the melanophores contract completely (fig. 1, c). The time required for the completion of the contraction is a function of the concentration of pituitary extract. In order to obtain comparative results, the standardization tests are all carried out at a constant temperature. We have made all tests at 22°C.

THE THERMOSTAT

A double wooden tank lined with galvanized iron and having a capacity of about 200 litres serves as a reservoir in which water is kept at 22.4°C. by means of an electric radiator bulb and a mercury thermoregulator similar to the De Khotinsky type.

A horizontal ⅜ inch brass shaft (*s*, fig. 2) carrying four adjustable paddles passes through a stuffing box (*b*) at one end of the reservoir and is turned by means of a 10 inch wheel and motor (*m*). A faucet at one end of the reservoir connects with a large warming stage as shown in figure 2.

The warming stage—20 by 11 by 5 cms.—is made of galvanized iron with a glass bottom. It carries three stender dishes which are fitted snugly into the top by means of rubber rims at the edges of the holes (*rr* fig. 3). Inlet and outlet pipes are attached at either end, *a* and *b*, and at one end there is an additional opening to carry a thermometer, *t*. The water circulates through the warming stage from the reservoir and the temperature, as measured within the stage, is easily kept at 22°C. for hours at a time. The solutions to be tested are brought into the three stender dishes and are kept covered except while an observation is in progress. The degree of contraction in the melanophores is best observed with a small lens; the end point of the reaction is easily seen with the naked eye.

THE STANDARD SOLUTION

Since the new method depends upon the time required for a complete contraction in a group of melanophores by a solution of unknown concentration it seemed important to select for comparison as a standard contracting solution some substance which (1) was readily obtainable in a very pure condition and (2) had a constant effect upon the melanophores. Potassium chloride answers both requirements admirably. It can be obtained (Merk's Blue Label) in a highly purified condition and solutions of identical concentrations are easily prepared by titration with $AgNO_3$. Furthermore, we have never found a melanophore which, when taken from a living fish, failed to contract completely in a 0.1N solution of KCl.

If the melanophores are immersed in a pure solution of 0.1N KCl even for a short time they are seriously injured. In 0.1N NaCl, on the other hand, the melanophores remain fully expanded and reversible by KCl or other contracting stimulus for several hours. The time required for a complete contraction in 0.1N KCl may be lengthened at will by diluting the KCl with NaCl. This procedure, above a definite concentration of NaCl also gives a less injurious combination than either pure salt solution.

In our standardization tests we have selected a mixture of 2.5 parts 0.1N NaCl + 1 part 0.1N KCl at 22°C., neutral to neutral red, as a standard solution with which to compare unknown strengths of pituitary extract. The 0.1N solutions were made up with glass distilled water and Merk's Blue Label salts. They were kept in old glass bottles.

PRELIMINARY TESTS

A series of preliminary experiments were carried out to determine the sensitivity of the melanophores to varying concentrations of KCl. The procedure was as follows:

A fish was gently removed *by hand* from the laboratory aquarium[3] and, after rinsing off with tap and distilled water and 0.1N NaCl solution, thirty to fifty scales were removed simultaneously from one side of the fish by means of a small scalpel, and immediately immersed in 0.1N NaCl solution. In the course of fifteen to twenty minutes the melanophores were fully expanded. Half a dozen adjacent scales selected from the dorsal region of the fish were now carefully freed by separating them with forceps and dissecting needles. These six scales were then removed from the NaCl solution and rapidly placed in position on the dipping device, figure 4. They were then simultaneously immersed in the standard solution and in the course of thirty seconds to a minute the melanophores began to contract. The scales were carefully watched and grouped accord-

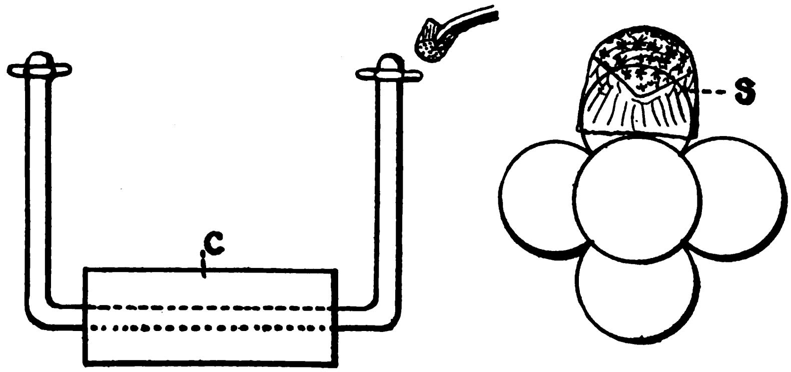

FIG. 4. THE DIPPING DEVICE

A large cork, *c*, carries a U-shaped glass rod. The ends of the rod are rounded and carry wing-like projections on which the scales are placed as shown in the detail in end view at the right, *s*. A scale is shown about to be placed in position on the dipper. The surface tension of the solution adhering to the scales keeps them in place on the dipper. The rounded ends of the rods prevent mechanical injury to the scales when being transferred from one solution to another, as shown in figure 3.

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[3] A number of fish were always kept in the laboratory at room temperature, in a small aquarium. The bottom of the aquarium was covered with white sand and, since the fish adapted themselves to the light bottom, the melanophores at the beginning of any series of experiments were always contracted.

ing to the pairs which required the same length of time for a complete contraction of all the melanophores. From a set of six scales it is almost always possible to select one pair, and frequently two, in which the melanophores contract in the same time. After the contraction was completed the paired scales were returned, *one pair at a time*, to 0.1N NaCl, whereupon the melanophores again expanded completely. This procedure thus gave pairs of physiological units which were known to behave identically under uniform conditions. By returning the paired scales to two different solutions, one the standard and the other a solution with more or less KCl than the standard solution, and observing the time required for a complete contraction of the melanophores in the two solutions the limits of sensitivity to differences in KCl concentration were established.

The concentration of KCl in the standard solution is 0.028_6N. A solution of 2.65 parts NaCl + 1 KCl in which the KCl has a concentration of 0.027_4N was indistinguishable from the standard; but 2.8 parts NaCl to 1 part KCl (KCl = 0.026_2N) showed a consistently *longer* contraction time than the standard. Within this region of concentration a difference of the order 0.003N could be detected by the use of matched pairs of scales.[4]

STANDARDIZATION OF PITUITARY EXTRACT

In the standardization of pituitary extract it is not necessary to use matched pairs of scales for the preliminary determination of the strength of the unknown solution. Approximate values may be obtained by comparing the contraction time in the melanophores of three adjacent scales taken directly from the 0.1N NaCl solution with the contraction time of three other scales selected from the same region and immersed in the standard solution.[5] So long as the unknown pituitary solution causes a distinctly more rapid contraction than the standard solution, all the melanophores of all three scales in the pituitary solution will contract before any scale in the standard solution shows complete contraction of the melanophores. The extent to which this approximation may be carried depends largely upon practice in selecting comparable scales by their propinquity, size and degree of pigmentation (number of melanophores). The method has an automatic check, however, since, as soon as the selection becomes faulty, the contraction times will no longer fall into two sharply divided groups, corresponding to the two sets of scales, but will overlap. Further comparisons of the standard and unknown solutions must then be made by using matched pairs of scales as described above until the reaction time of three successive pairs is the same.

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[4] It should be born in mind that this surprising degree of sensitivity on the part of the melanophores obtains only within a relatively limited series of concentrations. If the concentration of KCl is *increased* too much we approach the region of minimum contraction time.

[5] Separate samples of the standard solution should be used for making the preliminary tests and the final tests against the solutions containing the drug of unknown strength.

"Pituitrin" (Parke, Davis and Company) contains as a preservative trichlor—tertiary—butyl alcohol ("chloretone") nearly to saturation. This interferes somewhat with the standardization of the marked samples by the melanophore method. It might be supposed that an equivalent concentration of "chloretone" could be added to the standard solution of KCl and NaCl but this has not proven satisfactory. In practice we have diluted commercial pituitary extract with NaCl so as always to have solutions of a concentration of 0.1N NaCl.

In commercial practice we suggest that for the standardization of a given quantity of drug, solutions should be made up for immediate use, wherever possible, without preservative of any sort. As a uniform standard for pituitary extract we suggest an aqueous solution which, when diluted with equal parts 0.2N NaCl, will contract of group of expanded Fundulus melanophores in the same length of time as is required for the contraction of a comparable group of melanophores in the standard KCl solution.

CRITICISM OF THE NEW METHOD

The application of pituitary extract in obstetrical practice appears to have caused a prejudice in favor of the use of the uterus method for standardization. We have discussed above some of the reasons for doubting the validity of the uterus test. The objection may be advanced that even though the melanophores are functionally modified smooth muscle cells their relation to the mammalian uterus is so remote that the action of pituitary extract on the melanophores would give little indication of the action of the same extract on the mammalian, and especially the human, uterus. This objection, in so far as it applies to the guinea-pig uterus, has been met by empirical tests. Furthermore commercial samples of pituitary extract which were supposedly of the same physiological strength were found, by the use of the melanophore method, to vary widely. The explanation of this variability we believe may be found in the relatively far greater accuracy that may be obtained by the use of the melanophore technique.

A second objection might be advanced against the use of KCl in the standard solution. This salt, while easily procured in exceedingly pure form, is not chemically related, so far as we now know, to the pressor principle of pituitary extract. Furthermore, the effect of KCl and pituitary extract might be due to two quite unrelated physiological causes.[6] The relative sensitivity to KCl and pituitary extract might, therefore, vary independently. Whereas we have not yet investigated this point in complete detail our experiments have thus far shown that groups of melanophores which react slowly to KCl show a corresponding lack of sensitivity to pituitary extract.[7]

The accuracy of any method for drug assaying must, on last analysis, depend upon the number of variables involved. The technique proposed in this paper eliminates at least one important source of error, i.e., individual variation in the test animals. It furthermore affords an opportunity for an exact, quantitative, simultaneous, control experiment. These are the two chief theoretical advantages.

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[6] Dr. Barbour and I found that, in its action on the melanophores, ergotoxine reversed the normal pressor effect of epinephrine but not of KCl. (3, 4, '17).

[7] This objection might also be advanced against the use of histamine in the uterus method.

The practical advantages of hardy and inexpensive test animals combined with the relative simplicity and speed of technical manipulation are too obvious to require further comment.

In a subsequent paper we shall deal with the variations of market samples of pituitary extract and the degree of accuracy which may be obtained by the melanophore method of drug assaying.

Samples of pituitary extract were kindly furnished by the H. K. Mulford Company and the Parke, Davis Company research laboratories.

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CRITICAL NOTES ON THE PRESENT STATUS OF THE LENS-PROBLEM.

E. I. WERBER.

1. Introduction.

The present communication is an attempt to arrive at a common understanding of the nature of the difficulties that stand in the way of the solution of a problem to which for over a quarter of a century have been devoted the best efforts of a number of biological observers.

One phase of this problem, although at one time rather near solution, has since, owing to—apparently unavoidable—technical difficulties of the experimental method, become ever more complex. In the following it is my intention to show that this complexity is an artificial one and that it practically disappears on careful sifting of evidence. Owing to this seeming complexity, however, an originally correct interpretation has lately been giving way to one that not only is unwarranted, but has already occasioned even some very improbable and fruitless phylogenetic speculations (cf. for instance Becher, '12).

Anticipating to return to the subject of this communication in a more comprehensive publication in the near future I hope I may be pardoned for incompleteness in considering the pertinent literature at the present time. Only such contributions can here be considered, as from my point of view seem to have the most significant bearing on the problem.

2. The Origin of the Primary Lens—in Ontogeny.

As well known Spemann ('01, '03) was the first to furnish experimental evidence for the correctness of the opinion—advanced simultaneously also by Herbst ('01)—that the lens of the vertebrate eye is dependent in its origin and differentiation upon the contact of the optic vesicle with the supra-ocular epidermis from which it arises. It will be recalled also that this view was contested by Mencl ('03, '08), King ('05) and Stockard ('10) and that Spemann himself has in later papers ('08, '12) modified his opinion and abandoned his generalizations. He now thinks that more extended experiments in which various methods were employed on several frog species prove that in some species the lens cannot develop if the optic vesicle fails to come into contact with the overlying epidermis while in one species the epidermis is capable of giving rise to lentiform bodies and even well-differentiated, unmistakable lenses without such contact.

Such conditions would, indeed, be so contrary to expectation that it well behooves us to inquire into the validity of Spemann's self-contesting evidence as well, as of the evidence brought forth by Mencl, King and Stockard for the independent development of the lens.

I have already (Werber '16*c*) pointed out some possible sources of error which would invalidate the conclusions of these authors and on re-reading the entire series of Spemann's interesting papers on the subject I have been struck by the complete absence of any real counter-evidence against the conclusions of his earlier ('01, '03) excellent work.

Spemann's first experiments were performed on *Rana fusca* and consisted in destroying, by pricking with a heated needle or the galvanocauter, the right (presumable) optic pit (foveola optica).[1] As a result he found that no lens was formed, if the entire optic pit had been destroyed or, if deeply buried remnants of the latter which developed into diminutive optic cups failed to reach the epidermis. If, however, such a rudimentary optic cup did come into contact with the epidermis, a lens developed secondarily from the latter.

I am inclined to think that these results practically solved the problem, and for this reason it may perhaps be regarded as unfortunate that Spemann's well-warranted generalizations have been the subject of ill-founded criticism by Mencl ('03). For while the latter was very aptly met by Spemann ('03), it apparently occasioned him to enter upon a great many experiments on the subject which, though beautifully conceived and skillfully executed, have, owing to the pitfalls of a treacherous material, yielded fallacious results.

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[1] For a description of this earliest discernible eye primordium cf. Eycleshymer ('93 and '95) and Froriep ('06).

The first experiments which have occasioned Spemann ('07) to modify his views were performed on *Rana esculenta.* They consisted in the excision of the "right anterior half of the brain primordium" from the wide open medullary plate.[1] As a result he observed that a lens has developed in spite of the absence of an eye on the side operated upon. Like results were obtained also when the optic pit was destroyed at the same stage of development with a heated needle. The results were apparently entirely independent of the method of operation. In both cases, however, they differed from those obtained in *Rana fusca,* in which, as we have seen, no lens was formed when the optic anlage was at this stage destroyed by pricking with a heated needle, while the excision experiment in this species, according to Spemann ('07) "ergab ein sehr unsicheres Resultat." In one out of four examined embryos Spemann ('12) observed "ein kleines Bläschen, welches eine Linsenanlage sein könnte."

Why such divergent results in experiments on two species of the same frog genus with the same methods and at the same stage of development? Spemann concludes from his experiments on *Rana esculenta* that in this species the "lens-forming cells" of the embryonic epidermis are capable of independent development in the absence of an optic vesicle, while *Rana fusca* lacks this ability for self-differentiation.

The same experiments on *Bombinator pachypus* (at the same stage of development) gave less definite results. While no lens developed in this species on the side operated upon, Spemann observed in some few instances structures which he could not with certainty identify. Experiments on embryos of the same species at a later stage of development, *i. e.*, removal of the optic vesicle, the overlying epidermis being previously raised up and reflected from it, gave also ill-pronounced results. In a number of instances Spemann observed in such embryos on the side operated upon a thickening of the epidermis in the region where a lens normally should have developed, which he is inclined to consider as an abortive attempt at lens-formation. From these experiments Spemann tentatively concludes that *Bombinator* in regard to the power of independent lens-formation occupies a sort of intermediate position between *Rana fusca* which lacks this ability and *Rana esculenta* which, according to him, possesses it in a decidedly marked degree.

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[1] In my 1916c paper several, otherwise wholly insignificant, errors have unfortunately crept in into the references made to these excision experiments in *Rana esculenta* and to King's pricking experiments. In both cases it should read "optic pit" or "optic anlage" instead of "optic vesicle."

Are these conclusions justified? I am strongly inclined to think that they are not, the results on which they are based being very inconclusive. Moreover, on careful examination of the excision experiments in *Rana esculenta* the impression is gained that some fragments of optic substance were left after the excision of the anterior half of the primordium of the brain hemisphere. Thus, for instance, in his 1912 paper in Fig. 6 the smaller one of the two observed lentoids is in close apposition to a "ganglion." May not this ganglion rather be a small group of retinal cells? In Figs. 8 and 9 small groups of tapetum nigrum cells may be observed, and Spemann (p. 22) admits "dass vom Augenbecher vielleicht kleine Reste vorhanden seien." In Fig. 8 the tapetum-cells are very close to the lentoid, while Fig. 7 also suggests that dispersed tapetum nigrum was responsible for the origin of its "Linsenbläschen." Instead of concluding, as does Spemann, that the lentoids of Figs. 6 and 9 are due to dispersion of "lens-forming cells" it might perhaps be safer to think that in these experiments fragments of potential optic-cup substance were dispersed and stimulated the differentiation of lentoids by chancing to come into contact with the epidermis.

Again, in Fig. 10 representing a cross-section through an embryo in which the anterior part of the anlage of the right brain hemisphere has been destroyed by a heated needle, the lentoid observed is in close proximity to a rudimentary optic cup (oc′, Fig. 10), which clearly demonstrates the fact that the attempted elimination of the anterior part of the brain primordium may often be illusory, fragments of optic-pit substance remaining after the operation. According to Spemann ('12, pp. 24 and 25) it is easier to eliminate the entire optic anlage in *Rana fusca* than in *R. esculenta.* He attributes this to a difference in the "Konsistenz" between the embryos of the two species. "So ist wohl das Resultat zu erklären, dass der Defekt bei den allerdings nicht sehr zahlreichen Versuchen dieser Art meist entweder zu gross wurde, im ersteren Fall also vom rechten Auge ein Rest erhalten blieb, im letzteren die Linsenanlage mit zerstört wurde."

In my estimation the divergent results obtained both by pricking with a heated needle and by excision in both species would rather seem to point to differences in the location of the optic pits in the neurulae of the two species at the same stage of development. Thus, in *Rana esculenta* they are probably more diffuse and extend more laterally than they are in *R. fusca.* Accordingly, if at this stage the excision of the anterior half of the brain primordium be attempted, it may happen that a small fragment of the optic pit may remain, as in this species it may extend even laterally from the medullary fold. Granting, however, that fragments of potential optic-cup substance do sometimes remain after the operation in this species—and Spemann admits that both in text and in the figures—it is no longer difficult to understand that in such embryos lentoid structures and even well-differentiated lenses may be formed on the eyeless side owing to the "lentogenic stimulus"[1] from the remnant of optic substance on the epidermis.

Spemann does not seem to have considered the results obtained from these operations as very conclusive, if as much can remain of the brain anlage after its attempted elimination as "ein Fragment des Tapetum nigrum," "der vorderste Teil der Vorderhirnanlage, . . . ein dorsales Stückchen Zwischenhirn mit Epiphyse und Plexus chorioideus, jedenfalls entstanden aus lateralen Partien der Medullarplatte, endlich ein Stückchen der linken Hälfte des Mittelhirns" ('12 p. 28). For, not content with these results he sought confirmation in experiments in which several other methods were employed.

Thus, adopting Lewis's ('04) method, he removed (by excision) in *R. esculenta* the optic vesicle after having previously raised up and reflected the overlying epidermis. The latter was then affixed in its place where it healed and in two embryos gave rise to "ein deutliches Linsenbläschen" in the absence of the optic cup. Spemann considers these lens-buds as evidence of the ability for independent lens formation in *R. esculenta.*

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[1] Cf. Werber '16c.

The validity of this conclusion, however, seems questionable to me in view of a well-nigh uncontrollable source of error which Spemann himself has pointed out ('12, p. 42). For he states expressly that the lower stratum of the epidermis (the "Sinnesschicht") adheres so firmly to the eye vesicle that on attempting to separate the epidermis from the latter usually the upper layer (the "Deckschicht") is raised up, while the lower one remains attached to the optic vesicle. Regardless of Spemann's skill in such delicate operations and the precautions he has taken to separate the entire epidermis from the optic vesicle it was apparently impossible always to avoid minute fragments of the latter remaining attached to it. And the two cases in which he obtained "independent" lens-buds have possibly resulted from just such an unsuccessful operation, while in the embryos in which no such structures were recorded on the side operated upon the operation was apparently faultless.

Spemann assumes that in his unsuccessful experiments of this series, *i. e.*, where no "independent lenses" were recorded, the "lens-forming cells" of the lower stratum of the epidermis were removed with the optic vesicle to which they remained attached. I can see no valid reason for the assumption of cells predetermined to form lenses. The often-raised argument of the cyclopean eye in which the lens is formed from epidermis that normally does not develop into this structure, and Lewis's ('04, '07a and b) experiments in which the formation of lenses from strange ectoderm and even from ectoderm of another species was demonstrated to be possible—an experiment successfully repeated by Spemann himself by transplanting the bared optic vesicle of *Rana esculenta* under the ventro-abdominal epidermis of *Bombinator*—speak very decidedly against this assumption.[1]

Moreover, why should just the species *Rana esculenta* form such a strange exception as to possess such so early specialized regions of the epidermis? I am under the impression that the apparent fallacy of Spemann's conclusions from this series of experiments is most likely due to the circumstance that while he detected an important source of error, he unfortunately, mistook the nature of the error. For had he considered the possibility that fragments of the optic vesicle too small to be detected with the binocular dissecting microscope and even so minute as not to be able to differentiate into histologically discernible structures, may suffice to induce the lentogenic reaction in the epidermis to which they remained attached, he would probably have considered the "unsuccessful" experiments of this series as successful and vice versa. Accordingly, instead of believing that in the two "successful" cases the "lens-forming cells" had not (or not entirely) been removed, he might have concluded that owing to the above-mentioned difficulty some remnants of optic substance have stimulated the development of the lens-buds demonstrated in Figs. 37, 38 and 38a.

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[1] Mencl ('08, Fig. 3) described an "intracerebral eye" (intracerebral cyclopia) whose lens is derived from the epithelium of the mouth. The ability of this part of the ectoderm to give the "lentogenic reaction" is demonstrated also by the lentoids of the mouth which I have recorded (Werber '16c).

The results of other experiments performed by Spemann ('12) which may now be examined would also seem to lend support to his former views rather than to his present ones. One of these experiments consisted in transplanting abdominal epidermis over the bared optic vesicle in *Rana esculenta*. The flap of epidermis used for the purpose was previous to its transplantation "von etwa anhaftenden Mesodermzellen sorgfältig gereinigt." The result was negative—no lenses or lens-like structures were formed by the strange epidermis owing, as Spemann thinks, to the circumstance that the "primären Linsenbildungszellen" were contained in the flap of supra-ocular epidermis which had been removed. However, four cases were recorded in which the epidermis transplanted over the eye presented an appearance different from that of the epidermis of the immediate surroundings. And in Fig. 63 there is "eine deutliche Linsenanlage (L) vorhanden, ein kleines, dickwandiges Epithelbläschen, welches mit der transplantierten Rumpfhaut über dem Auge innig verbunden ist." ('12, p. 55). Spemann mentions also another embryo of this series in which he observed an indication of lens-formation on the side operated upon (Fig. 58), but assumes that in both cases "primäre Linsenbildungszellen" remained attached to the optic vesicle owing to faulty technique in the removal of the supra-ocular epidermis.

Might we not with at least just as much justification assume that in the embryos in which no lenses were formed from the transplanted epidermis this was due to lack of immediate contact between the optic vesicle and the epidermis, because mesodermal cells were attached to the latter? For, regardless of the most painstaking care I think it is impossible to be sure that the epidermis has been cleaned of these cells entirely with the aid of such low magnifications as even the highest power lenses of the binocular dissecting microscope. It is, besides, difficult to understand why the optic vesicle of this species should be able to induce lens-formation from ventro-abdominal epidermis of *Bombinator* and not from the abdominal epidermis of an embryo of its own species.

Whatever one might think of the results of these experiments, they certainly cannot be regarded as conclusive in favor of Spemann's present views.

Two more series of experiments on *Rana esculenta* may now be considered the results of which Spemann interprets in favor of the independent development of the lens in this species. The method employed in these experiments was autoplastic transplantation and the part transplanted was the "primären Linsenbildungszellen."

In the first (a) series a more or less rectangular piece of supra-ocular epidermis was detached during or immediately after the closure of the medullary tube and turned about 180°, care being taken that no fragments of the optic vesicle be left on it. In the second (b) series the operation differed only in one point, viz., that in detaching the supra-ocular epidermis a fragment of the optic vesicle remained attached to it and owing to the inversion of the piece of epidermis (by turning it about 180°), it became transplanted into a posterior region (otic capsule).

In the experiments of the first (a) series great technical difficulty was encountered and only in four cases was the separation of the epidermis from the optic vesicle "anscheined einwandsfrei." In three of these cases the eye of the operated side had "eine deutliche Linse," while in the fourth case where, owing to the operation, the otic labyrinth came to lie between the epidermis and the eye, the latter lacked a lens. No lens, however, developed in any of these embryos caudally from the eye, as might have been expected, had that part of the epidermis ("primäre Linsenbildungszellen") been capable of giving rise to a lens by self-differentiation.

These cases (although so few in number) point decidedly, I think, to the inability of supra-ocular epidermis in *R. esculenta* to differentiate a lens in the absence of a stimulus from the eye vesicle. Spemann, however, prefers the tentative explanation "dass nach Auslösung einer Linsenbildung durch's Auge die spontane Entwicklung einer zweiten Linse aus den primären Linsenbildungzsellen unterbleibt." He is inclined to consider even the possibility "dass bei diesen Experimenten die Linsenbildungszellen nicht weit genug nach hinten gebracht worden waren und dass entweder sie selbst oder ihre nächste Umgebung die Linse des stehengebliebenen Auges geliefert haben." As support for the latter possibility he regards the observation, "dass die Linse in allen 3 Fällen im hintersten Winkel des etwas deformierten Augenbechers liegt und einmal sogar etwas in die Länge gezogen ist" ('12, p. 64). Is it not more probable that this distorted relation between the lens and the optic cup is due to the very fact that the latter was deformed owing to the operation? From my observations on many teratophthalmic *Fundulus* embryos I know that this is very often the case in deformed eyes.

One cannot escape the impression that Spemann by considering such possibilities and by regarding "diese Versuche . . . als misslungen und die Frage als unentschieden" has—apparently unconsciously—missed the only obvious conclusion, viz., that *Rana esculenta* possesses no early predetermined lens-forming cells and that a stimulus from an optic cup or from some of its parts is necessary to induce the differentiation of a lens from the epidermis in embryos of this species as much as in *Rana fusca*.

Let us now consider the second (b) series of these experiments in which, as will be recalled, a fragment (the tip, "Kuppe") of the optic vesicle was left attached to the inverted epidermis.

Observations were made on seven embryos operated upon in this manner. In all of them the anterior eye fragment lacks the lens, while the posterior one in five embryos stimulated the formation of a lens which "*kann* so vollkommen entwickelt sein wie die normale" (p. 67). In one of these embryos the posterior eye fragment possesses even two lenses which Spemann is inclined to regard as being due to mechanical separation of the "Linsenmaterial" into two parts, although it would seem more probable that the duplication of the lens in this case may (owing to the operation) be due to mechanical histolysis of the eye fragment which thus came into contact with the overlying epidermis in two places.

From these observations Spemann (p. 67) concludes that "die weitgehende Determination der primären Linsenbildungszellen von *Rana esculenta,* welche sie zu selbstständiger Entwicklung befähigt, bringt es wohl mit sich, dass die übrigen Epithelzellen, auch die der nächsten Umgebung, nicht mehr imstande sind, auf einen Reiz des Augenbechers mit Linsenbildung zu antworten."

These conclusions cannot be considered as warrantable. Moreover, the results permit of an interpretation which, I believe, is founded upon a much greater probability. It must be remembered that at the stage of development at which the operations were performed, it is only the tip ("die Kuppe") of the eye vesicle that is protruding from the head. If that protruding part be removed, the epidermis transplanted over the resulting gap may, owing to the latter, fail to come into contact with the deeper part of the eye even on subsequent growth of the latter owing to the obstruction formed by mesenchyme cells which may grow into the gap before such contact can be effected. Spemann's Fig. 67*b* very forcibly suggests just this possibility. On examining the anterior eye fragment of this figure one can observe that it is not in contact with the epidermis. More significant, however, is the observation in its lumen of a blood vessel and of some mesenchyme cells which also fill the space between it and the epidermis.

Our interpretation of these apparently vexing cases receives further support from the very fact that in the (a) series of these experiments where the "Kuppe" of the optic vesicle had not been removed, the latter was able to stimulate the formation of lenses, because there being no gap between it and the epidermis transplanted over it, there was no chance for an obstruction of its contact with the latter by ingrowing mesenchyme. It is, besides, needless to say that it is difficult to understand just why Spemann in the experiments in which the epidermis was transplanted over a defective eye vesicle assumes a sharp predetermination to form lenses and not so, if transplanted over an uninjured eye vesicle.

Summarizing briefly *we find that critical sifting of the results of Spemann's experiments on Rana esculenta discloses no warrantable evidence whatsoever for the presence in this frog species of an early predetermined lens-forming part of the epidermis capable of differentiation into a lens without the stimulus from an optic vesicle or at least a fragment of it.*

Practically all of these experiments were performed by Spemann also on *Bombinator pachypus*. The results obtained in this species differed according to Spemann ('07, '12 ff) from those obtained in the pricking experiments in *Rana fusca* and also from the above-noted results in *Rana esculenta*. They indicate, Spemann believes, that *Bombinator* in regard to the ability to form a lens by self-differentiation occupies a position intermediate between *Rana fusca* and *Rana esculenta.*

It is evident, I believe, that were this really so, *i. e.*, if the supra-ocular epidermis of *Bombinator* possessed a certain degree of this ability and only needed the stimulus from the optic vesicle as a complementary aid for the differentiation of a lens, a serious stumbling block would here confront every attempt at the solution of the lens-problem.

Fortunately, however, Spemann's observations seem to call for an entirely different interpretation of the results.

Thus in the excision experiment (right foveola optica) the latter prove very decidedly, I believe, that no lens can be formed in the absence of an optic anlage or on its failure to come tinto contact with the overlying epidermis. For, out of the forty-six embryos operated upon in twenty of them in which an optic fragment remained (owing to incomplete excision), a lens developed on the side of operation. Of the remaining twenty-six cases one is uncertain, for here a structure was observed which might perhaps be a deformed lens-bud, in twenty cases neither an optic cup nor a lens can be observed, while in five cases the optic cup is so small that there was no contact with the epidermis. According to Spemann, however, in two of the latter cases the condition is not clear, one of the embryos possessing (on the side of excision) near the brain "ein dickwandiges Bläschen mit kleinem Lumen," which Spemann is unable to identify, while in the other case the region of epidermis which should have furnished the lens, is "kaum merklich verdickt."

The only warranted conclusion from these results is: *The contact of at least a fragment of the optic anlage is necessary to induce the formation of a lens from the overlying epidermis.* It is unwarranted however, to conclude, as does Spemann (12, p. 38), ". . . dass Bombinator pachypus zwar auch primäre Linsenbildungszellen besitzt, welche von den Epidermiszellen der Umgebung verschieden und zur Umbildung in die Linse vorbereitet sind, dass diese Zellen aber der Mitwirkung des Augenbechers bedürfen, um in Aktion zu treten, zum mindesten in viel höherem Masse als die Linsenbildungszellen von R. esculenta."

The results of other experiments in this species also point decidedly to the incorrectness of Spemann's conclusions. Thus the removal of the optic vesicle during or immediately after the closure of the medullary folds resulted only in a thickening ("Wucherung") of the epithelium in the region where normally the lens should have arisen. In two cases, however, small remnants of the optic vesicle developed into diminutive optic cups lacking a lens, because mesenchyme had grown in between them and the epidermis. Lentoid structures (two) were observed only in one case (Figs. 41*a* and *b*, L'), which are probably due to a stimulus from very minute remnants of the optic vesicle.

These experiments would again seem to *prove only that,* as Lewis ('04, '07*a* and *b*) and Le Cron ('07) have found, *a stimulus from the optic vesicle on the epidermis of sufficient duration is necessary for the latter to give rise to a lens.* They do not prove, however, the presence in this species of early predetermined lens-forming cells requiring the stimulus from an optic vesicle only as a supplement for full differentiation into a lens.

On transplanting ventro-abdominal epidermis over the bared optic vesicle in this species Spemann recorded as a result that in none of the sixteen examined embryos a lens developed from the strange epidermis. From these results he concludes that either the optic vesicle cannot stimulate abdominal epidermis to the formation of a lens or that this part of the epidermis is incapable of responding to such a stimulus.

Here again, however, the same objection is unavoidable which we have raised against the conclusion from the results of the same experiments in *Rana esculenta,* namely that regardless of the experimenter's care mesenchyme cells attached to the transplanted epidermis obstructed a contact of the latter with the optic vesicle.[1]

Very clear ("eindeutig") results were obtained in the experiments in which, as in *Rana esculenta*, a flap of the skin of the head including the supra-ocular epidermis was turned about 180°. In the first (a) series in four out of eight "einwandsfreie" cases the eye cup was not in contact with the epidermis and, naturally, lacked a lens. In the other four cases there was such contact—and the eye possessed a lens.

Accordingly: *There are no lens-forming cells, but epidermis that normally does not give rise to a lens will differentiate into a lens, if brought into contact with the optic vesicle.*

The second (b) series of these experiments (a fragment, the "Kuppe," of the optic vesicle transplanted with the turned epidermis) yielded results very similar to those of the corresponding experiments in *Rana esculenta.*

With but one exception the posterior eye fragment always obtained a lens, which, as Spemann rightly adds, could not have developed in the absence of this fragment of optic-cup substance.

The anterior eye fragment, however, obtained no lens in fifteen out of the twenty examined embryos, while a lens or only a thickening of the epidermis opposite the eye fragment was observed in five cases. In thirteen of the embryos which had no anterior lens Spemann accounts for its lack by the ingrowth of mesenchyme into the space between the optic-vesicle fragment and the epidermis, widened by the expansive growth of the otic capsule which, owing to the transplantation came to occupy this anterior position. He is unable, however, to suggest an explanation for the lack of this lens in two cases, "wo kein Grund für ihr Ausbleiben zu erkennen ist" (p. 73).

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[1] It is significant that in one out of the four cases in which he reports to have observed a "Wucherung" opposite the eye "eine zusammenhängende Schicht sehr dotterreicher Zellen" (p. 60) was conspicuous in that interspace. That shows that it is apparently futile to attempt to clean the abdominal epidermis of all mesenchyme cells. While it is difficult to know just how to interpret these "Wucherungen," they might possibly be due to mechanical distortion during the operation.

From these experiments Spemann ('12, p. 77) concludes that the optic vesicle of *Bombinator* is capable of exerting a specific stimulus not only on the lens-forming cells, but also on other parts of the head epidermis.

This conclusion is, I think, only partly correct, the assumption of "primäre Linsenbildungszellen" being both unwarranted and unnecessary. For leaving out of consideration the two cases (which I should not wish to pre-judge), where no anterior lens was formed while it should have been expected, we can conclude only that its presence in the several other cases was due to the specific stimulus from the anterior eye fragment.

Briefly, the results of this series of experiments also contradict Spemann's opinion of the intermediate position of *Bombinator* between *Rana fusca* and *R. esculenta* with regard to the ability of independent differentiation of the lens.

In *all of Spemann's work* I find only *a confirmation of his initial results and strong support for the generalizations which he made in 1901 and 1903, but no counter-evidence whatsoever that would justify his present* ('*12*) *opinion.*

Other evidence for the independent development of the lens brought forth by Mencl ('03, '08), King ('05) and Stockard ('10) is no less illusory.

Mencl ('03) described the head of an anophthalmic component of an anadidymus in *Salmo salar* which possessed two laterally located lenses although there could be observed "keine Spuren von Augenblasen, ja nicht einmal von Anlagen derselben." Both of these lenses were in close apposition to the deformed brain, the larger one even having, owing to its growth, formed a pit in the latter by pressure. Both lenses, however, were so situated that their derivation from lateral parts of the head epidermis was beyond doubt. What is responsible for the origin of these lenses? Mencl suggested (p. 337) that "der, diese zwecklose, wie durch Erinnerung der Epidermiszellen auftauchende Linsenbildung auslösende Faktor ist die Vererbung."

A much more reasonable and, I think, the only correct, interpretation of this case was offered by Spemann ('03) who concluded (p. 464) that the optic vesicles "oder genauer ihr für die Linsenbildung allein in Betracht kommender retinaler Teil nur scheinbar fehlen, indem die Partie der Hirhwand, welcher die Linsen angelagert sind, nichts anderes ist, als die nicht abgegliederte . . . Retina."

Although this interpretation was later abandoned by Spemann "unter dem Druck neuer Tatsachen" ('12, p. 3), my experience with teratological material—and Mencl's case is a teratological one—forces me to lend it unreserved support.

For the sake of clearness in presenting my point of view (based largely on teratological data) the following brief recapitulation of the present stage of our knowledge on the morphogenesis of monsters may be permitted.

It was repeatedly noted by pathologists and teratologists and notably also by Mall ('09) that examination of malformed embryos often discloses evidence of destruction, dissociation and shifting of tissues or parts of the embryo. On examination of a great many experimentally produced monsters in *Fundulus heteroclitus* I ('16*a* and *b*) was able not only to confirm these observations, but also to demonstrate some remarkably striking cases of the effects of such dissociation of parts of the early embryonic primordium and to account for the causes of this process which I have termed blastolysis. This "blastolytic action of the chemically modified environment is . . . a morphogenetic principle common to all terata. . . . Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter" (Werber '16*b*, p. 569).

It is this destruction of tissues, the subsequent elimination of parts destroyed and the resulting dissociation and shifting of parts surviving that in experiments in which eggs are subjected to a chemical modification of the environment, brings about the weirdest malformations of the developing embryos.

All of these deformities are thus clearly *due to a defect* (*or defects*) *of a blastolytic nature.* Not only is this the case in experiments in which the modification of the environment employed is a chemical one, but also, if a physical, *e. g.*, thermic, modification be employed. For, in experiments (not yet published), in which the eggs of *Fundulus heteroclitus* were subjected to the action of a temperature much below the normal, some terata and particularly ophthalmic monsters resulted. The latter are due to loss at an early embryonic stage of parts of ophthalmoblastic material and to like damage sustained by the anterior part of the potential head. Thus anophthalmia results from such loss by the earliest brain primordium of the whole or nearly whole ophthalmoblastic material.

In some anophthalmic embryos such blastolytic optic-cup fragments or even small, fairly well differentiated optic-cups may on microscopic examination of sections be observed either enclosed in the brain between both hemispheres (intracerebral cyclopia, as it were, cf. Mencl '08, Figs. 2 and 3) or in a lateral position as a part of the brain. Such "concealed" remnants of the optic cup may often lack the tapetum nigrum and the histological character of retina, owing to early destruction of some groups of cells which potentially corresponded to the lacking layers of the retina, and may thus give the appearance of a part of the brain. If it happens to come into contact with the epidermis, such "a part of the brain" will stimulate the differentiation of a lens from it.

This, undoubtedly, is exactly the condition in the case described by Mencl in 1903. I have many times observed unmistakable optic cups which lacked some of the histological characteristics of retina and whose structure was very much like that part of the "brain" in Mencl's case (cf. Mencl '03, Figs. 2, 4 and 5, and also '08, Fig. 1), into which the larger one of the two lenses has, as it were, burrowed in. That part of the "brain" is undoubtedly a "verkappte" retina, as Spemann had once suggested.

This, I think, disposes of the first, but most troublesome, case described by Mencl. It might perhaps be added that the asymmetric position of the two lenses[1] in the head of this anophthalmic anadidymus component could not be accounted for by "hereditary reminiscences," while chance contact of remnants of dissociated ophthalmoblastic material of both sides with the epidermis does account for the origin of both lenses as well as for their asymmetrical position. Neither of them has arisen from predetermined, lens-forming cells—"durch Erinnerung," but from indifferent ectoderm in response to stimuli from remnants of potential optic cups on the epidermis.

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[1] "Die linke liegt mehr dorsal und zugleich caudalwärts von der rechten" (Mencl, '03, p. 331).

The other "independent" lenses and lentoids described by Mencl ('08) can even much easier be demonstrated to be due to the same origin—blastolysis and subsequent contact of fragments of optic cup substance with the ectoderm. From his description of sections of the embryos underlying the Figs. 4, 5, 6, 7 and 8 the reader will easily gather that in these cases fragments of optic-cup substance[1] have been profusely dispersed and in places of contact with ectoderm gave rise to lenses or lentoids. Mencl did not appreciate the full significance of his own observations, for referring to such an optic fragment ("Pigmentanhäufung," Fig. 5*X*, he says: "Was dies Gebilde bedeutet, ***kann ich nicht entscheiden—das Vorhandensein des Pigments jedoch, sowie die retinaartige Anordnung der Zellen in der benachbarten Hirnwand***[2] lässt die Meinung entstehen, dass es sich dabei um irgend ein Rudiment einer atypisch und selbstständig zur Entwicklung gelangten Augenkomponente handelt" (p. 447). It is, indeed, difficult to understand just why, in spite of these observations, Mencl claims that in these embryos "die selbstständige Entwicklung der Linsen über jeden Zweifel erhaben ist" (p. 447).

I have recently examined sections of a number of deformed Salmonid embryos (of another species) with such "independent" lenses. The publication of the observations made on this material being reserved for a future publication, it may suffice to state that the conditions noted in several of these embryos bore a very striking resemblance to those in the embryos lately described by Mencl ('08). Here, too, subjective interpretation might easily lead to the conclusion that the "eyeless" lenses arose by self-differentiation of the ectoderm. But careful observation and rigid analysis of the noted relations of parts to each other leaves no doubt that all these lenses are products of stimuli from dispersed optic-cup substance.

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[1] "Pigmentanhäufung," "Pigmentbläschen," "Pigmentfleck," "eine homogene, schwarze, längliche Pigmentmasse, welche in die Falte der retina-artigen Hirnwand hineinragt" (Mencl '08, p. 447).

[2] My own italics.

In a *Fundulus* monster described by me in a former paper (Werber '16*c*) there were no eye defects, but owing to a special method optic-cup substance was very profusely dissociated and dispersed through a large part of the head. Owing to this condition a great deal of the head ectoderm and even the epithelium of the mouth, infected, as it were, with such fragments of the optic anlage, responded by the formation of a great number of lentoids. Many more monsters resulting from the employment of the same method were examined in sections and they show very similar conditions. In other experiments in which the method employed was more destructive various eye defects such as monophthalmia or synophthalmia or anophthalmia resulted. On examination of sections through these embryos not only lentoids, but well-differentiated, "eyeless" lenses were frequently observed and in nearly every one of these cases some more or less obvious traces of optic-cup substance can be observed in their immediate neighborhood.

In the same paper I have pointed out that the "independent" lenses which Stockard ('09, '10) described in teratophthalmic *Fundulus* embryos have also undoubtedly resulted from contact of ectoderm with such blastolytic fragments of the eye anlage.

On that occasion I have also called attention to a very probable source of error in King's ('05)[1] experiments in view of which the evidence she brought forth for the independent development of the lens in *Rana palustris* in contradiction to Lewis's ('04) unmistakable evidence to the contrary, appears to be illusory.

Experiments of other observers (Bell '06 and '07 and Ekman '14) also yielded results which decidedly contradict the idea of the independent development of the lens. And a very beautiful demonstration of the "lentogenic reaction" of the epidermis to a stimulus from an eye fragment was recently furnished by Wachs ('14, p. 430 and Figs. 46, 47, 48 and 49).

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[1] Cf. the footnote on p. 221 of this paper.

This author transplanted in well-advanced larvæ (hind legs developed) of *Triton tæniatus*, in which the eye was at the time of operation as fully developed as in the adult, a piece of the iris from the eye of one larva into the otic capsule of another larva of the same age. As a result he could in one case observe that a strand of epidermal epithelium which, owing to the operation, had grown in from the edge of the wound to the otic capsule, has given rise to "ein rundes Lentoid . . . mit konzentrisch gelagerten Linsenfasern . . .."

From all that has been said so far we may conclude that *there exists no valid evidence for the possibility of the origin of the lens by self-differentiation.* On the contrary, *observation in the normal development of the eye, evidence from teratophthalmic cases and all experimental evidence point to the correctness of Herbst's* ('01) *and Spemann's* ('01) *conclusion that the lens of the vertebrate eye depends in its development and differentiation from ectodermal epithelium upon a specific, apparently chemical, stimulus from the optic vesicle.*

This stimulus might perhaps be in the nature of an enzyme action by contact. Spemann ('05, '12), who recognizes the dependence of the development of the lens upon this specific stimulus in some species, assumes the possibility of a specific secretion by the optic cup or rather by the retinal part of the latter, to which, as he believes, may also be due the "regeneration" of the lens from the iris of the fully developed amphibian eye. While this hypothesis appears to have received strong support from the beautiful experiments of his pupil Wachs (*l. c.*), it seems to me perhaps premature to speak of a secretion in this case. For, aside from other considerations, the fact ascertained by Wachs (*l. c.*) that the supposedly secreted substance is not conveyed by the blood of the animal to any other part of the body would seem sufficient to indicate that the retina, as Wachs himself concludes, has no such endocrine function. Nor is Wachs's assumption of a secretion that "bleibt auf das Auge und seine nächste Umgebung beschränkt" (p. 446) justified by the results of his numerous experiments.

There is, moreover, good reason to believe that the capacity for the "lentogenic stimulus" is not at all restricted to the retinal layers of the eye cup. For evidence is not lacking that the tapetum nigrum is also capable of inducing the development of a lens (or lentoid) when in contact with ectodermal epithelium. Thus, as pointed out above (p. 235), some of the "independent" lenses recorded by Mencl ('08) very clearly owe their origin to contact of ectoderm with tapetum nigrum fragments ("Pigmentbläschen," "Pigmentanhäufungen." "Pigmentmasse"). It was also mentioned that in a considerable number of *Fundulus* monsters in my possession "independent" lenses and lentoids can be demonstrated to be due to this "enzyme action" of the tapetum nigrum. This ability of the pigment layer of the optic cup is also suggested by some of Spemann's ('12) and Stockard's (*l. c.*) figures.

Whatever the nature of this action may be, whether or not we agree to regard it as an enzyme action, as I am inclined to do, it seems evident that all layers of the optic cup are capable of it. Even the iris epithelium which genetically is also a part of the optic cup may also be capable of this action.

3. THE ORIGIN OF THE SECONDARY LENS—IN "REGENERATION."[1]

Bearing in mind this apparent ability of the embryonic ectoderm for lens formation—by "enzyme action," it may perhaps no longer be difficult to account, at least theoretically, for its secondary formation—"regeneration"—from the iris after the exstirpation of the primary lens from the fully developed eye of the amphibian larva or even the adult.

An attempt in this direction has already been made by Wachs (*l. c.*) who accounts for this phenomenon as a reaction of the epithelium of the iris to the "secretion" of the retina. Although I find it difficult to accept the evidence for this secretion, I believe that Wachs's idea is correct in the main. The secondary formation of the lens—from the epithelium of the iris—is probably a response (reaction or complex of reactions) to a chemical stimulus just as much as its primary development from the supra-ocular epidermis of the early embryo.

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[1] I am not inclined to regard the formation of the lens from the epithelium of the iris as a case of true regeneration in the precise meaning of that term. *From the histogenetic point of view* we must say that *this matrix (the iris) gives rise to the lens for the first time* and thus it *generates*, but does *not re*generate, it. *Considering*, on the other hand, the *secondary lens in relation to the whole visual organ or to the entire body of the animal*, it is difficult to deny that *we are here dealing with* a case of what is commonly regarded as *regeneration* ("Ersatz", "Nachbildung", "Nachwachsen", "Wiederwachstum"). Without wishing, therefore, to decide on the fitness of the term in this connection, I am employing it only in quotation marks.

But, it may well be asked, if there is no such secretion as assumed by Wachs, what is the nature of the chemical stimulus and whence, from what source, or from what part of the eye does it issue? Two possibilities suggest themselves as an answer to this query. It may be imagined that, owing to the operation, the iris comes into temporary contact with the inner wall of the optic cup, from which it might thus receive the stimulus for the formation of a lens in the same manner as in the embryo the supra-ocular epidermis from the optic vesicle. Such contact due to a collapse of the optic cup[1] may result from the methods of operation employed by Wolff ('95) and the other experimenters (with the exception of Wachs). However, I am not inclined to give serious consideration to this possibility, as this contact of the pupillary edge of the iris is not necessary for the "regeneration" of a lens, for in Wachs's experiments the method of operation excludes it altogether. The experiments of the latter author, however, do not, as he believes, exclude another possibility which to me seems well worthy of careful consideration.

Wachs (*l. c.*, pp. 416–426) has raised the question whether the iris is capable of forming a lens without any outside stimulus ("aus sich heraus"). He was led to consider this possibility by the results of some of his experiments, in which a fragment of the iris, implanted into the posterior chamber of an eye deprived of its lens "regenerated" a lens, even if it did not heal on to the iris. This lens may even be better differentiated than the lens simultaneously "regenerated" by the latter.

To decide whether the fragment of iris in these cases formed a lens under the influence of a secretion from the retina or—"aus sich heraus," he transplanted (in a number of experiments) a fragment of the iris under the skin of the head. The result was negative, as the piece of iris disintegrated and was resorbed.

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[1] According to a statement by Müller ('96) no lens is formed by the iris after extraction of the primary lens, if the vitreous body is injured. Were this statement correct, no lens could arise from the iris owing to its contact with the optic cup by collapse of the latter. It is evident, however, that Müller's observation is incorrect, for in Wolff's (*l. c.*) and in some of Fischel's ('00 and '02) experiments in which a lens or lentoids did form from the iris, the vitreous body was undoubtedly injured, since the whole optic cup was damaged.

Wachs concluded from this result that the site of this transplantation was apparently unfavorable and he, therefore, in the next experiments transplanted a fragment of the iris into the otic labyrinth, after first removing a part of the latter. The following results were obtained:

In several cases where the fragment was "small," *i. e.*, consisted of iris only without retinal cells or with some very few of the latter, no lens-like structures were formed. In a number of instances, however, where the fragment was "larger," *i. e.*, containing more retinal cells, lentoids were formed (apparently from the iris), while in several other cases the transplanted fragment was transformed into a small eye with a lens, which latter Wachs considers as formed from the fragment of iris. However, in a number of instances (pp. 425, 426–428) where the transplanted fragment contained many retinal cells and was in good condition ("trotz guter Erhaltung") no lens-like structures were formed.

These results are, obviously, inconclusive. They certainly do not permit of Wachs's conclusion that the lentoids and lenses of the "positive" cases owe their origin to a stimulus from a secretion of the retinal cells (carried with the transplanted fragment of iris). For, why could not such effect of the retinal "secretion" be observed in the "negative" cases in spite of considerable retina? From these results I can read no definite, unmistakable, answer to the question which they were to answer.

For the following reasons, however, I should regard the query as a very pertinent one.

We know that the retina (Spemann, Lewis, Le Cron, Bell, Ekman, Werber and others) can furnish the stimulus for the formation of a lens from an epithelial derivative of the ectoderm (even epithelium of the mouth—Mencl, '08, and Werber, '16*c*) by contact with it. Besides being capable of furnishing the stimulus for this "lentogenic reaction" the retina (an ectodermal derivative) is according to Fischel's ('00, '02) and my (Werber, '16*c*) observations capable also of responding to such a stimulus—the lentoids of the retina. Whence does this stimulus issue in the case of the formation of the retinal lentoids? Considering the fact that the retinal cells can both furnish the "lentogenic" stimulus and respond to it, it would certainly seem not far fetched to assume that the stimulus for the formation of the lentoids by the cells of the retina issues from themselves. In this case the retinal cells react to their own "lentogenic enzyme." Both the conditions for furnishing the stimulus and the latent potency of responding to such a stimulus are known to be present in the retina. May not this double capacity underly also the formation of the lens from the iris?

To answer the latter question it would be necessary to find out whether the iris besides its known potency of forming a lens (Wolff, Müller, Fischel, Wachs, and others) is also capable of furnishing the "lentogenic stimulus," if in contact with epithelium (or some other ectodermal derivative). Unfortunately, however, the question cannot be answered definitely at the present time.

There exists only one experiment (by Wachs, *l. c.*, p. 430 and Figs. 46, 47, 48 and 49) which suggests this possibility, but the conditions in this experiment are not quite clear. A fragment of the iris was in this case (No. 39) transplanted into the cartilaginous capsule of the otic labyrinth. This fragment stimulated the formation of a lens (Fig. 49) from a strand of epithelium that had grown in from the edge of the wound. It is uncertain, however, whether in this case the "lentogenic" stimulus issued from the iris or perhaps from retinal cells contained in the transplanted fragment. For while on p. 429 Wachs states that this fragment was "ein Stück der oberen Iris" and that "nach 28 Tagen . . . war das Stück mit Goldpigment noch deutlich sichtbar," on p. 447 (in the summary) he refers to it as "ein Stück des Auges" and in the record (p. 423) this case (No. 39) is enumerated among those in which "ein Stück des oberen Augenteiles" was transplanted into the labyrinth. Wachs himself, however, does not state whether he assumes that in this case the regenerated epithelium formed the lens under the influence of retinal cells or of the iris.

There is no reason, however, why the latter possibility should be excluded. It is certainly not excluded by those experiments of Wachs in which a fragment of the eye cup containing both iris and retina (cf. Fig. 50) transplanted "dicht unter die Haut" has failed to stimulate lens-formation from the latter, but instead has so altered it that it gave the appearance "einer kleinen Cornea." For in these cases the failure to induce the lentogenic reaction might have to be attributed to the retinal as well to the iris-part of the transplanted fragment; it may, however, be due to the circumstance that the skin of the relatively old larvae was no longer capable of responding to a "lentogenic stimulus." The latter possibility may be implied from the (above-mentioned) case (pp. 237 and 241) in which owing to the operation an ingrowing strand of epithelium formed a lens under the influence of the transplanted fragment of the optic cup. For, as Wachs himself concludes, we are in that case (No. 39) dealing with "Abkömmlinge von Hautzellen, die, neugebildet und noch undifferenziert, offenbar die gleiche Fähigkeit der Linsenfaserbildung haben können, wie einst die junge Haut . . . " (*l. c.*, p. 430).

But while there is, as we see, no definitely known instance of lens-formation owing to a stimulus from the iris, there is, on the other hand, nothing that would contradict the assumption that the iris is capable of exerting such a stimulus on ectodermal derivatives. Moreover, the very fact that the other parts of the optic cup (retina and tapetum nigrum) can furnish the lentogenic stimulus would make it appear very probable that the iris which genetically is also a part of the optic cup (proper), is likewise possessed of the ability to induce the "lentogenic reaction."

Granting the correctness of this assumption (which eventually may be borne out by suitable experiments), we would in the case of the iris be confronted by the same conditions as in the case of the retina. It is known that the iris can form a lens, and if, as seems probable, it can also induce the formation of a lens from ectodermal epithelium, the assumption may be justified that in the formation of a lens from the pupillary edge of the iris, which is an ectodermal derivative, its cells respond to their own "lentogenic enzyme."

These conditions apparently obtaining in the formation of lentoids from the retina and very probably also in the "regeneration" (secondary formation) of the lens from the iris are in principle similar to the conditions obtaining in the primary (embryonic) formation of the lens, although the morphogenetic scheme of the latter cannot be fully applied to either the retinal lentoids or to the lens formed from the iris. The chief difference between the two modes of the morphogenesis of the lens is that while the origin of the "lentogenic stimulus" and the part responding to it are locally separate in the primary formation of the lens, they would—granting the correctness of our interpretation—coincide locally in the secondary formation of the lens from the iris or of a lentoid from the retina.

In order to fully understand the morphogenetic process underlying the "regeneration" of the lens from the iris (or of lentoids from the retina), it would also seem necessary to explain just how the "lentogenic stimulus," the latent capacity for which is probably possessed by the whole optic cup proper, is activated on the extraction of the primary lens. An answer to this query is, I believe, partly given by some of Fischel's ('02) experiments, which I shall review in the following.

The lens is so tightly enclosed by the iris that on its extraction slight injuries to the latter result. No matter how carefully the operation may be performed, slight, unnoticeable, lesions (abrasions?) of the cells of the pupillary edge of the iris may be unavoidable.[1] The significance of these, otherwise perhaps negligible, physical alterations of these cells of the iris resulting from the operation suggested itself to Fischel ('02, pp. 106–109 ff) from the observation of small lenses and lentoids formed not only by the pupillary edge but also from other parts of the iris. Being aware of accidental injuries to the eye cup and to the iris in some of his experiments he thought of a possible causal connection between the latter and such lenses or lentoids located in various parts of the iris. In order to test the correctness of his tentative interpretation he performed a number of experiments in which besides the exstirpation of the lens various parts of the iris were purposely injured. On microscopic examination of the results he was able to observe that small lenses or lentoids were formed from just such intentionally, and still discernibly distorted, parts of the iris. In several experiments Fischel observed besides the formation of a lens from the pupillary edge of the iris small double lenses, or twin (fused) lenses and in one case even three lenses in other parts of the latter. For the formation of these double (or multiple) structures Fischel, I think, correctly accounts by close proximity to each other of two (or more) places of injury.[1] Even the size of such lens-like structures he regards as dependent upon the extent (number of cells) of the injured area.

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[1] According to E. Uhlenhuth (quoted from Loeb '16) who cultivated fragments of the iris by Harrison's explantation method, it is to these abrasions that the loss of the pigment by the cells of the iris is due. Owing to this injury of the cells the pigment granules are liberated into the lumen of the optic cup where, as Wolff ('95) has observed, they may be absorbed by leucocytes.

The lens formed by the pupillary edge of the iris after exstirpation of the primary lens is usually as large and as well differentiated as the latter. This, according to Fischel, is due to the circumstance that on extraction of the lens a large area comes under the stimulus of the injury, and also to the fact that the pupillary margin of the iris being an epithelial fold, it affords the best means for the formation of a lens-bud (the "Knoten"—Wolff '95), the first step in which is always a folding of the corresponding part of the epithelium.

Another apparent confirmation of this "Reizhypothese" was furnished by the lentoids of the retina. The latter were observed by Fischel ('02) in many experiments in which owing to the extraction of the lens the eye bulb sustained accidental injuries. The experimental test—intentional injury of the retina—again gave positive results. The "retinal lentoids" could be demonstrated to be due to transformation of thus injured cells or groups of cells of the retina into such lens-like structures. The fact that only the latter and not fully differentiated lenses of large size can be formed by the retina on mechanical stimulation is, according to Fischel, accounted for by the small area of such stimulation and by the difficulty of forming a large fold.

This ability of the retinal cells to become transformed into lens-like structures Fischel regarded already in 1902 as no more surprising than, (as demonstrated by his experiments), that of any part of the iris, or of the epithelium of the skin in normal ontogeny. Already at that time he regarded it as a primary potency of all derivatives of the ectoderm. As the additional factor, however, necessary for this transformation of ectodermal derivatives he assumed an unknown stimulus which in the "regeneration" of the lens from the iris "*in jenen Alterationen zu suchen ist, welche das regenerierende Gewebe direkt durch den experimentellen Eingriff selbst erfährt* . . . von welchen die Zellen der Iris bei der Linsenextraktion betroffen werden" ('02, p. 106).

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[1] In some experiments of Wachs (*l. c.*) in which a fragment of the iris was introduced into the posterior eye chamber of another animal whose lens has been extracted, both the implanted fragment and the animal's own iris formed a lens. In some of these cases where the fragment coalesced with the iris, two more or less fused lenses were observed at the point of coalescence, one formed by the foreign fragment and one by the iris.

This unknown stimulus Fischel ('16) now regards as a chemical one from a secretion by the optic cup, or more specifically, from the retina which in conformance with Spemann and Wachs he also assumes. To the stimulus from this secretion he attributes the transformation of the supra-ocular epidermis into a lens in the embryo as well as the like transformation of the cells of any injured part of the optic cup (iris or retina).

While in the main I agree with Fischel by recognizing the necessity of an injury to that part of the iris or retina from which the secondary lens or lentoid arises, as proven, I am, as already stated, inclined to doubt the probability of a secretion by the eye cup as the additional factor involved in the stimulus for the formation of the secondary lens. Undoubtedly, with the present data at hand, it is difficult to deny that some chemical process is apparent in the morphogenesis of both the primary and the secondary lens. This process, however, may, as was pointed out in the preceding pages, be due to a substance (a "lentogenic enzyme") apparently contained by all parts of the eye cup already in the stage of the optic vesicle or even earlier. The activation, however, of this "enzyme" seems in the case of the "regeneration" of the lens to be in some way due to the injury of those cells of the iris which subsequently become transformed into lens fibers.

What would yet remain to be explained is the nature of the relation of the injury of the cells to the activation of their own "lentogenic enzyme." On this point, however, I should venture no opinion at the present time.

One apparent objection to our interpretation may yet be considered.

It is known from experiments by Wolff ('03) that if a part of the iris be removed by cutting (iridectomy) without removing the lens, the iris will regenerate but form no lens-like structures. Wachs (*l. c.*) attempted to account for this fact by the assumption of a secretion from the lens which would inhibit the formation of another lens. The results of some of his experiments, he believes, substantiate this conclusion. In these experiments the lens was removed and subsequently the lens of another animal of the same or a related species was introduced into the eye. In no case in which the foreign lens healed into the eye was a lens "regenerated." This result, however, permits of another interpretation, in which no resort is made to the improbable assumption of the "antisecretion" by the lens.

Indeed, Wachs's own ingenious experiments performed for the solution of this particular part of the lens-problem would seem to disprove rather than to prove the assumption of a secretion. For they show that the "regeneration" of a lens is inhibited only when the (smaller) lens implanted into the eye, heals into it, *i. e.*, comes into close contact with the iris ("so dass die verengerte Iris sich ihr ringsum dicht anschliesst . . . ," p. 404). If, however, such contact is not effected—"liegt jedoch die aus dem jüngeren Tiere implantierte kleinere Linse mehr oder weniger in der vorderen oder hinteren Kammer, so wird eine Regeneration eingeleitet."

Wachs has ascertained beyond doubt that the inhibition is in these cases due not only to the mechanical effect of the contact, but very apparently also to a chemical action. While, however, he strongly inclines to the belief that the chemical action is due to a secretion, I think it can easily be shown also in all other of his experiments by which the matter was tested, that this chemical action is conditioned by contact of the implanted lens with the iris.

These important experiments of Wachs, strengthened further by some interesting results of Fischel's ('02) experiments[1] would seem, however, to leave no doubt that the lens contains something, some substance that apparently inhibits the formation of another lens from the iris which would invariably occur in its absence.[1] This substance, however, again need not necessarily be secreted, in order to neutralize the action of the "lentogenic enzyme." Just what the nature of this substance may be, is, of course, altogether a matter of conjecture. It might perhaps be imagined as an "antibody" ("antigen") contained in the peripheral part (the epithelial cells) of the lens and the realization of its inhibiting action may be due to close contact of that part of the lens with the epithelium of the iris.

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[1] Fischel exstirpated the lens and replaced it by small spherical fragments of the potato tuber. Whenever this "imitation lens" was large enough to fit the pupilla, no "regeneration" of a lens from the latter took place. If, however, its diameter was smaller than that of the pupilla, partial "regeneration" of the lens or at least an "attempt" towards it was noted, complete "regeneration" being impossible owing to mechanical obstruction by the potato fragment. This experiment clearly demonstrates that the extraction of the lens eliminates not an indifferent body, but one with a specific, *inhibiting*, action.

This "antibody" being, just like the lens, a product of the "lentogenic reaction," we may perhaps in the case of the vertebrate eye eventually have a striking example of some of the factors (synthetic enzyme action—Loeb, '16) concerned not only with development and growth, but also with the limitation of the latter[2] for the attainment of proper size relations, and with the maintenance of a chemical equilibrium indispensible for the undisturbed existence of a structure or an organ.[3]

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[1] Fischel ('16) has recently furnished another interesting example of the apparent chemical action of the lens which he also considers as a "secretion." He transplanted in larvae (about 3 cm. large) of *Salamandra maculosa* the exstirpated lens under the skin of various parts of the head or trunk. As a result he observed that, while the lens underwent a gradual dedifferentiation and eventual absorption, the skin above the transplantate showed remarkable changes. The unicellular glands (the cells of Leydig) disappeared from the corresponding region of the skin, which, owing to the morphological changes, by its appearance suggested a similarity to "frühe Entwicklungsstadien des Hautepithels" (p. 37). Eventually this region of the skin became transparent and was very similar to a fully differentiated cornea.—Similar observations were, as mentioned above (p. 242), recorded also by Wachs (*l. c.*) on transplanting parts of a fully differentiated optic cup under the skin.

[2] The fact that the "regenerating" lens does not grow indefinitely (as it might, if it were a benign tumor—regarded as such at one time by Fischel, '00), but is limited in its growth to attain just the size to fit the pupilla, has recently occasioned J. Loeb ('16) to raise the question regarding the factors limiting growth. According to our assumption it might perhaps be imagined that every tissue (or structure) elaborates during its development a substance (an "antibody") which inhibits its growth beyond certain limits. In this case both growth and its automatic limitation would be conditioned by the same factors of development.

[3] Fischel ('16) concludes from his experiments that the already fully differentiated ens needs the chemical influence of the eye for its normal (undisturbed) existence.

Osborn Zoölogical Laboratory,
Yale University, October 4, 1917.

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ART. XI.—*A Century of Zoology in America;* by WESLEY R. COE.

This article is intended as a brief survey of the development of zoology in America, and no attempt is made to give a general history of the science. There are numerous accounts in several languages of zoological history in general, among them being W. A. Locy's "Biology and its Makers." Brief outlines of the history of zoology may be found in many zoological and biological text-books.

For the history of American zoology the reader is referred to Packard's report on "A Century's Progress in American Zoology," published in the American Naturalist, (**10**, 591, 1876), to Packard's "History of Zoology," published in volume **1** of the Standard Natural History (pp. lxii to lxxii, 1885); to G. B. Goode's "Beginnings of Natural History in America,"[1] and "Beginnings of American Science,"[2] and to H. S. Pratt's Manual of the Common Invertebrate Animals (pp. 1-9), 1916. In Binney's "Terrestrial Air-breathing Mollusks of the United States" (1851) is a chapter on the rise of scientific zoology in the United States which well describes the zoological conditions in the early part of the century, while numerous monographs and papers give the history of the investigations on the various groups of animals or on special fields of study.

Brief biographical sketches of the most distinguished of our older Naturalists—Wilson, Audubon, Agassiz, Wyman, Gray, Dana, Baird, Marsh, Cope, Goode and Brooks are given in "Leading American Men of Science," edited by David Starr Jordan, 1910.

The developmental history of zoology in America falls naturally into four fairly well marked periods, namely:—1, *Period of descriptive natural history,* previous to 1847, embracing the early studies on the classification and habits of animals, characteristic of the zoological work previous to the arrival of Louis Agassiz in America. 2, *Period of morphology and embryology,* 1847-1870, during which the influence of Agassiz directed the zoological studies toward problems concerning the relationships of animals as indicated by their structure and developmental history. 3, *Period of evolution,* 1870-1890, when the principle of natural selection received general recognition and the zoological studies were largely devoted to the applications of the theory to all groups of animals. 4, *Period of experimental biology,* since 1890, during which time have occurred the remarkable advances in our knowledge of the nature of organisms through the application of experimental methods in the various branches of the modern science of biology.

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[1] Proc. Biol. Soc. Washington, 3, 35, 1886.

[2] Ibid, 4, 9, 1888. Both of these papers are reprinted in Ann. Rept. Smithsonian Inst., 1897, U. S. Nat. Mus., Pt. 2, pp. 357-466, 1901.

AMERICAN ZOOLOGY IN 1818.

At the beginning of the century which this volume commemorates, the accumulated biological knowledge of the world consisted mainly of what is to-day called descriptive natural history. The zoological treatises of the time were devoted to the names, distinguishing characters and habits of the species of animals and plants known to the naturalists of Europe either as native species or as the results of explorations in other parts of the world. This required little more than a superficial knowledge of their general anatomical structures.

The naturalists of those days had no conception of the life within the cell which we now know to form the basis of all the activities of animals and plants, nor had they even the necessary means of studying such life. The compound microscope, so necessary for the study of even the largest of the cells of the body, was not adapted to such use until 1835, although the instrument was invented in the 17th century. With the perfection of the microscope came a period of enthusiastic study of microscopic organisms and microscopic structures of higher animals and plants. It was not until twenty years after the founding of this Journal that the cell theory of structure and function in all organisms was established by the discoveries of Schleiden and Schwann.

At the beginning of the century there was great zoological activity in Europe, and particularly in France. Buffon's great work on the Natural History of Animals had recently been completed, Cuvier had only one year before published his classic work in comparative anatomy, "Le Regne Animal," and Lamarck's "Philosophie

Zoologique" had then aroused a new interest in classification and comparative anatomy from an evolutionary standpoint. É. Geoffroy St.-Hilaire was at the same time supporting an evolutionary theory based on embryonic influences resulting in sudden modifications of adult structure. These epoch-making discoveries and theories gained a considerable following in France, Germany and England, but seem to have had little influence on the zoological work of the following half century in America.

The science of zoology as understood to-day is commonly said to have been founded by Linnæus by the publication of the modern system of classification in the tenth edition of his "Systema Naturæ" in 1758. The influence of Linnæus aroused an interest in biological studies throughout Europe and stimulated new investigations in all groups of organisms. Such studies as related to animals naturally followed first the classification and relationship of species, that is, systematic zoology, and then led gradually into the development of the different branches of the subject, as morphology, comparative anatomy, physiology, and embryology, which eventually were recognized as almost independent sciences.

Of these sciences systematic zoology, which has come to mean the classification, structure, relationship, distribution and habits, or natural history, is the pioneer in any region. Thus we find in our new country at the time of the founding of this Journal in 1818, only sixty years after the publication of Linnæus' great work, the beginning of American zoology taking the form of the collection and description of our native animals.

It is true that many of our more conspicuous and easily collected animals were described long before the opening of the 19th century, but this is to be credited mainly to the work of European naturalists who had made expeditions to this country for the purpose of studying and collecting. These collections were then taken to Europe and the results published there. We thus find in the 12th edition of Linnæus descriptions of over 500 American species, about half of which were birds. As an illustration of the extent to which some of these works covered the field even in those early days may be mentioned a monograph in two quarto volumes with many beautifully colored plates on the "Natural History of the rarer Lepidopterous Insects'' of Georgia. This was published in London in 1797 by J. E. Smith from the notes and drawings of John Abbot, one of the keenest naturalists of any period.

During the early years of the 19th century, however, economic conditions in our country became such as to give opportunity for scientific thought. Educated men then formed themselves into societies for the discussion of scientific matters. This naturally led to the establishment of publications whereby the papers presented to the societies could be published and made available to the advancement of science generally. The most influential of these was the Journal of the Philadelphia Academy of Natural Science, which was established in 1817, and was devoted largely to zoological papers. The Annals of the New York Lyceum of Natural History date from 1823, and the Journal of the Boston Society of Natural History from 1834. The Transactions of the American Philosophical Society in Philadelphia and the Memoirs of the American Academy of Arts and Sciences in Boston also published many zoological articles.

In these publications and in this Journal, which was founded in 1818, appear the descriptions of newly discovered animal species, with observations on their habits.

The number of investigators in this field in the first quarter of the 19th century was but few, and most of these were compelled to take for the work such time as they could spare from their various occupations.

Gradually the workers became more numerous until about the middle of the century zoology was taught in all the larger colleges. The science thereby developed into a profession.

For some years the studies remained largely of a systematic nature, and embraced all groups of animals, but long before the close of the century the attention of the majority of the ever increasing group of zoologists was directed into more promising channels for research and there came the development of the sciences of comparative anatomy, physiology, embryology, experimental zoology, cytology, genetics, and the like, while the systematists became specialists in the various animal groups.

But the work in systematic zoology remains incomplete and many native species are still undescribed or imperfectly classified. It is perhaps fortunate that a few faithful systematists remain at their tasks and tend to keep the experimentalists from the disaster which might well result from the unwitting confusion of their species.

PERIOD OF DESCRIPTIVE NATURAL HISTORY.—PREVIOUS TO 1847.

Of the few American naturalists whose writings were published toward the end of the eighteenth century and at the beginning of the nineteenth the names of William Bartram (1739-1823), Benjamin Barton (1766-1815), Samuel Mitchill (1764-1831), William Peck (1763-1832), and Thomas Jefferson (1743-1826), require special mention. Bartram's entertaining volume describing his travels through the Carolinas, Georgia and Florida, published in 1793, contains a most interesting account of the birds and other animals which he found.

Barton wrote many charming essays on the natural history of animals, but was more particularly interested in botany. Mitchill's most important works include a history of the fishes of New York (1814), and additions to an edition of Bewick's General History of Quadrupeds. The latter, published in 1804, contains descriptions and figures of some American species and is the first American work on mammals.

Peck has the distinction of writing the first paper on systematic zoology published in America. This was a paper on new species of fishes and was printed in 1794. He is also well known for his work on insects and fungi.

Jefferson in 1781 published an interesting book describing the natural history of Virginia, and during his presidency was of inestimable service to zoology through his support of scientific expeditions to the western portions of the country.

Previous to Agassiz's introduction of laboratory methods of study in comparative anatomy and embryology in 1847, American naturalists generally confined their attention to the study of the classification and habits of the multitude of undescribed animals and plants of the region.

Such studies were naturally begun on the larger and more generally interesting animals such as the birds and mammals, and although many of these were fairly well described as to species before the opening of the 19th century, little was known of their habits. The natural history of our eastern birds first became well known through the accurate illustrations and exquisitely written descriptions of Alexander Wilson (in 1808-1813). Bonaparte's continuation of Wilson's work was published in four folio volumes beginning in 1826.

In 1828 appeared the first of Audubon's magnificent folio illustrations of our birds. These were published in England, with later editions of smaller plates in America. Nuttall's Manual of the Ornithology of the United States appeared in 1832-1834.

The second work on American mammals appeared in the second American edition of Guthrie's Geography, published in 1815. The author is supposed to have been George Ord, although his name does not appear. In 1825 Harlan published his "Fauna Americana: Descriptions of the Mammiferous Animals inhabiting North America." This was largely a compilation from European writers, particularly from Demarest's Mammalogie, and had little value.

In 1826 Amos Eaton published a small "Zoological Text-book comprising Cuvier's four grand divisions of Animals: also Shaw's improved Linnean genera, arranged according to the classes and orders of Cuvier and Latreille. Short descriptions of some of the most common species are given for students' exercises. Prepared for Rensselaer school and the popular class-room." "Four hundred and sixty-one genera are described in this text-book. They embrace every known species of the Animal Kingdom." This is a compilation from European sources with a few American species of various groups included. On the other hand, Godman's Natural History, in three volumes (1826-1828), was an illustrated and creditable work. Such was also the case with Sir John Richardson's Fauna Boreali Americana of which the volume on quadrupeds was published in England in 1829. The other volumes on birds, fishes and insects appeared between 1827 and 1836. Audubon and Bachman's beautifully illustrated "Quadrupeds of North America" was issued between 1841 and 1850.

About 1840 several of the states inaugurated natural history surveys and published catalogues of the local faunas. The reports on the animals of Massachusetts and New York are the most complete zoological monographs published in America up to that time. This is particularly true of DeKay's Natural History of New

York published between 1842 and 1844 in beautifully illustrated quarto volumes.

The leader in the systematic studies in the early part of the century was Thomas Say, who published descriptions of a large number of new species of animals, particularly reptiles, mollusks, crustacea and insects. Say's conchology, printed in 1816 in Nicholson's Cyclopedia, is the first American work of its kind. This was reprinted in 1819 under the title "Land and Fresh-water Shells of the United States." In 1824-1828 appeared the three volumes of Say's American Entomology.

The prominent position held by Say in the zoological work of this period is illustrated by the following paragraph from Eaton's Zoological Text-book (1826, p. 133). "At present but a small proportion of American Animals, excepting those of large size, have been sought out . . . And though Mr. Say is doing much; without assistance, his life must be protracted to a very advanced period to afford him time to complete the work. But if every student will contribute his mite, by sending Mr. Say duplicates of all undescribed species, we shall probably be in possession of a system, very nearly complete, in a few years." How different is the attitude of the zoologist of to-day who sees the goal much further away after a century's progress through the industry of hundreds of investigators.

During the period of Say's most active work he is reported to have "slept in the hall of the Philadelphia Academy of Natural Sciences, where he made his bed beneath the skeleton of a horse and fed himself on bread and milk."

Next to Say, the most active zoologist of the early part of the century was Charles Alexander Lesueur, who described and beautifully illustrated many new species of fishes, reptiles, and marine invertebrates. A memoir by George Ord, published in this Journal (**8**, 189, 1849), gives a full list of Lesueur's papers.

One of the most prolific writers of the period was Constantine Rafinesque, a man of great brilliancy but one whose imagination so often dominated his observations that many of his descriptions of plants and animals are wholly unreliable.

United States Exploring Expedition.—In 1838 a fortunate circumstance occurred which eventually brought

American systematic zoology into the front ranks of the science. This opportunity was offered by the United States Exploring Expedition under the command of Admiral Wilkes. With James D. Dana as naturalist, the expedition visited Madeira, Cape Verde Islands, eastern and western coasts of South America, Polynesia, Samoa, Australia, New Zealand, Fiji, Hawaiian Islands, west coast of United States, Philippines, Singapore, Cape of Good Hope, etc.

Of the extensive collections made on this four-years' cruise, Dana had devoted particular attention to the study of the corals and allied animals (Zoophytes) and to the crustacea. In 1846 the report on the Zoophytes was published in elegant folio form with colored plates. Six years later the first volume of the report on Crustacea appeared, with a second volume after two additional years (1854). These reports describe and beautifully illustrate hundreds of new species, and include the first comprehensive studies of the animals forming well-known corals. They remain as the most conspicuous monuments in American invertebrate zoology. Unfortunately the very limited edition makes them accessible in only a few large libraries. The other, equally magnificent, volumes include: Mollusca and Shells, by A. A. Gould, 1856; Herpetology, by Charles Girard, 1858; Mammalogy and Ornithology, by John Cassin, 1858.

Principal investigators.—Of the many writers on animals at this period of descriptive natural history, the following were prominent in their special fields of study:

Ayres, Lesueur, Mitchill, Storer, Linsley, Wyman. DeKay, Smith, Kirtland, Rafinesque and Haldeman described the fishes.

Green, Barton, Harlan, Le Conte, Say, and especially Holbrook, studied the reptiles and amphibia. Holbrook's great monograph of the reptiles (North American Herpetology) was published between 1834 and 1845.

Wilson, Audubon, Nuttall, Cooper, DeKay. Brewer. Ord, Baird, Gould, Bachman, Linsley and Fox, were among the numerous writers on birds.

Godman. Ord, Richardson, Audubon, Bachman, DeKay, Linsley and Harlan, published accounts of mammals.

On the invertebrates an important general work entitled "Invertebrata of Massachusetts; Mollusca, Crustacea, Annelida and Radiata" was published by A. A. Gould in 1841, which contains all the New England species of these groups known to that date.

Lea, Totten, Adams, Barnes, Gould, Binney, Conrad, Hildreth, Haldeman, were the principal writers on mollusks. The crustacea were studied by Say, Gould, Haldeman, Dana; the insects by Say, Melsheimer, Peck, Harris, Kirby, Herrick; the spiders by Hentz; the worms by Lee; the coelenterates and echinoderms by Say, Mantell and others.

The history of entomology in the United States previous to 1846 is given by John G. Morris in this Journal (1, 17, 1846). In this article F. V. Melsheimer is stated to be the father of American Entomology, while Say was the most prolific writer. Say's entomological papers, edited by J. L. Le Conte, were completely reprinted with their colored illustrations in 1859. The first economic treatise is that by Harris on Insects injurious to Vegetation, printed in 1841. This has had many editions. The Entomological Society of Pennsylvania was formed in 1842.

The establishment of this Journal gave a further impetus to the scientific activities of Americans in furnishing a convenient means for publishing the results of their work. In the first volume of the Journal, for example, are two zoological articles by Say and a dozen short articles on various topics by Rafinesque, the latter being curious combinations of facts and fancy. Most of the zoological papers appearing in its first series of 50 volumes are characteristic of an undeveloped science in an undeveloped country. They deal, naturally, with observational studies on the structure and classification of species discovered in a virgin field, with notes on habits and life histories.

Many of the papers are purely systematic and include the first descriptions of numerous species of our mollusks, crustacea, insects, vertebrates and other groups. Of these, the writings of C. B. Adams, Barnes, A. A. Gould and Totten on mollusks, of J. D. Dana on corals and crustacea, of Harris on insects, of Harlan on reptiles, and of Jeffries Wyman and D. Humphreys Storer on fishes are representative and important.

The progress of zoology in America during the first twenty-eight years of the Journal's existence, that is, up to the year 1846, is thus summarized by Professor Silliman in the preface to vol. **50** (page ix), 1847:

> "Our zoology has been more fully investigated than our mineralogy and botany; but neither department is in danger of being exhausted. The interesting travels of Lewis and Clark have recently brought to our knowledge several plants and animals before unknown. Foreign naturalists are frequently visiting our territory; and, for the most part, convey to Europe the fruits of their researches, while but a small part of our own is examined and described by Americans: certainly this is little to our credit and still less to our advantage. Honorable exceptions to the truth of this remark are furnished by the exertions of some gentlemen in our principal cities, and in various other parts of the Union."

During these 28 years the Journal had been of great service to zoology not only in the publication of the results of investigations but also in the review of important zoological publications in Europe as well as in America. There were also the reports of meetings of scientific societies. In fact all matters of zoological interest were brought to the attention of the Journal's readers.

THE INFLUENCE OF LOUIS AGASSIZ.

At the time of the founding of this Journal and for nearly thirty years thereafter descriptive natural history constituted practically the entire work of American zoologists. In this respect American science was far behind that in Europe and particularly in France. It was not until the fortunate circumstances which brought the Swiss naturalist, Louis Agassiz, to our country in 1846 that the modern conceptions of biological science were established in America.

Agassiz was then 39 years of age and had already absorbed the spirit of generalization in comparative anatomy which dominated the work of the great leaders in Europe, and particularly in Paris. The influence of Leuckart, Tiedemann, Braun, Cuvier and Von Humboldt directed Agassiz's great ability to similar investigations, and he was rapidly coming into prominence in the study of modern and fossil fishes when the opportunity to continue his research in America was presented. On arriving on our shores the young zoologist was so inspired with the opportunities for his studies in the new country that he decided to remain.

Bringing with him the broad conceptions of his distinguished European masters, he naturally founded a similar school of zoology in America. It is from this beginning that the present science of zoology with its many branches has developed.

It must be remembered in this connection that the great service which Agassiz rendered to American zoology consisted mainly in making available to students in America the ideals and methods of European zoologists. This he was eminently fitted to do both because of his European training and because of his natural ability as an inspiring leader.

The times in America, moreover, were fully ripe for the advent of European culture. There were already in existence natural history societies in many of our cities and college communities. These societies not only held meetings for the discussion of biological topics, but established museums open to the public, and to which the public was invited to contribute both funds and specimens. This led to a wide popular interest in natural history. It was therefore comparatively easy for such a man as Agassiz to develop this favorable public attitude into wide popular enthusiasm.

The American Journal of Science announces the expected visit of Agassiz as a most promising event for American Zoology (**1**, 451, 1846): "His devotion, ability, and zeal—his high and deserved reputation and . . . his amiable and conciliating character, will, without doubt, secure for him the cordial coöperation of our naturalists . . . nor do we entertain a doubt that we shall be liberally repaid by his able review and exploration of our country." We of to-day can realize how abundantly this prophecy was fulfilled.

In the succeeding volume (**2**, 440, 1846) occurs the record of Agassiz's arrival. "We learn with pleasure that he will spend several years among us, in order thoroughly to understand our natural history."

Immediately on reaching Boston, Agassiz began the publication of articles on our fauna, and the following year he was appointed to a professorship at Harvard. The Journal says (**4**, 449, 1847): "Every scientific man in America will be rejoiced to hear so unexpected a piece of good news." The next year the Journal (5, 139, 1848) records Agassiz's lecture courses at New York and Charleston, his popularity with all classes of the people and the gift of a silver case containing $250 in half eagles from the students of the College of Physicians and Surgeons.

The service of Agassiz to American zoology, therefore, consisted not only in the publication of the results of his researches and his philosophical considerations therefrom, but also, and perhaps in even greater degree, in the popularization of science. In the latter direction were his inspiring lectures before popular audiences and the early publication of a zoological text-book. This book, published in 1848, was entitled "Principles of Zoology, touching the Structure, Development, Distribution and Natural arrangement of the races of Animals, living and extinct, with numerous illustrations." It was written with the coöperation of Augustus A. Gould. The review of this book in the Journal (6, 151, 1848) indicates clearly the broad modern principles underlying the new era which was beginning for American zoology.

> "A work emanating from so high a source as the Principles of Zoology, hardly requires commendation to give it currency. The public have become acquainted with the eminent abilities of Prof. Agassiz through his lectures, and are aware of his vast learning, wide reach of mind, and popular mode of illustrating scientific subjects . . . The volume is prepared for the student in zoological science; it is simple and elementary in style, full in its illustrations, comprehensive in its range, yet well considered and brought into the narrow compass requisite for the purpose intended."

The titles of its chapters will show how little it differs in general subject matter from the most recent text-book in biology. Chapter I, The Sphere and fundamental principles of Zoology; II, General Properties of Organized Bodies; III, Organs and Functions of Animal Life; IV, Of Intelligence and Instinct; V, Of Motion (apparatus and modes); VI, Of Nutrition; VII, Of the Blood and Circulation; VIII, Of Respiration; IX, Of the Secretions; X, Embryology (Egg and its Development); XI, Peculiar Modes of Reproduction; XII, Metamorphoses of Animals; XIII, Geographical Distribution of Animals; XIV, Geological Succession of Animals, or their Distribution in Time.

A moment's consideration of the fact that all these topics are excellently treated will show how great had been the progress of zoology in the first half of the 19th century. The sixty years that have elapsed since the publication of this book have served principally to develop these separate lines of biology into special fields of science without reorganization of the essential principles here recognized. This remained for many years the standard zoological and physiological text-book, and was republished in several editions here and in England. Another popular book is entitled "Methods of Study in Natural History" (1864).

More than 400 books and papers were written by Agassiz, over a third of which were published before he came to America. They cover both zoological and geological topics, including systematic papers on living and fossil groups of animals, but most important of all are his philosophical essays on the general principles of biology.

One of Agassiz's greatest services to zoology was the publication of his "Bibliographia Zoologiæ et Geologiæ" by the Ray Society, beginning with 1848. The publication of the Lowell lectures in Comparative Embryology in 1849 gave wide audience to the general principles now recognized in the biogenetic law of ancestral reminiscence. As stated in the Journal (**8**, 157, 1849), the "object of the Lectures is to demonstrate that a natural method of classifying the animal kingdom may be attained by a comparison of the changes which are passed through by different animals in the course of their development from the egg to the perfect state; the change they undergo being considered as a scale to appreciate the relative position of the species." These "principles of classification" are fully elucidated in a separate pamphlet, and are discussed at length in the Journal (**11**, 122, 1851).

One of the most interesting of Agassiz's numerous philosophical essays, originally contributed to the Journal (**9**, 369, 1850), discusses the "Natural Relations between Animals and the elements in which they live." Another philosophical paper contributed to the Journal discusses the "Primitive diversity and number of Animals in Geological times" (**17**, 309, 1854). Of this systematic papers, those on the fishes of the Tennessee river, describing many new species, were published in the Journal (**17**, 297, 353, 1854).

Agassiz's beautifully illustrated "Contributions to the Natural History of the United States" cover many subjects in morphology and embryology, which are treated with such thoroughness and breadth of view as to give them a place among the zoological classics. The Essay on Classification, the North American Testudinata, the Embryology of the turtle, and the Acalephs are the special topics. These are summarized and discussed at length in the Journal (**25**, 126, 202, 321, 342, 1858; **30**, 142, 1860; **31**, 295, 1861).

The volume on the "Journey in Brazil" (1868) in joint authorship with Mrs. Agassiz is a fascinating narrative of exploration.

The conceptions which Agassiz held as to the most essential aim of zoological study are well illustrated in his autobiographical sketch, where he writes:[3]

> "I did not then know how much more important it is to the naturalist to understand the structure of a few animals, than to command the whole field of scientific nomenclature. Since I have become a teacher, and have watched the progress of students, I have seen that they all begin in the same way; but how many have grown old in the pursuit, without ever rising to any higher conception of the study of nature, spending their life in the determination of species, and in extending scientific terminology!"

It is not surprising, then, that under such influence the older systematic studies should be replaced in large measure by those of a morphological and embryological nature.

The personal influence of Agassiz is still felt in the lives of even the younger zoologists of the present day. For the investigators of the present generation are for the most part indebted to one or another of Agassiz's pupils for their guidance in zoological studies. These pupils include his son Alexander Agassiz, Allen, Brooks, Clarke, Fewkes, Goode, Hyatt, Jordan, Lyman, Morse, Packard, Scudder, Verrill, Wilder, and others—leaders in zoological work during the last third of the nineteenth century. Through such men as these the inspiration of

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[3] Louis Agassiz: his Life and Correspondence, by Elizabeth Carey Agassiz, p. 145, 1885.

Agassiz has been handed on in turn to their pupils and from them to the younger generation of zoologists.

The essential difference between the work of Agassiz and that of the American zoologists who preceded him was in his power of broad generalizations. To him the organism meant a living witness of some great natural law, in the interpretation of which zoology was engaged. The organism in its structure, in its development, in its habits furnished links in the chain of evidence which, when completed, would reveal the meaning of nature. Of all Agassiz's pupils, probably William K. Brooks most fittingly perpetuated his master's ideals.

PERIOD OF MORPHOLOGY AND EMBRYOLOGY. 1847-1870.

The new aspect of zoology which came as a result of the influence of Agassiz characterized the zoological work of the fifties and sixties, that is, until the significance of the natural selection theory of Darwin and Wallace became generally appreciated.

The work in these years and well into the seventies was largely influenced by the morphological, embryological and systematic studies of Louis Agassiz and his school. The structure, development, and homologies of animals as indicating their relationship and position in the scheme of classification was prominent in the work of this period. The adaptations of animals to their environment and the application of the biogenetic law to the various groups of animals were also favorite subjects of study.

The most successful investigators in this period on the different groups of animals include:—Louis Agassiz on the natural history and embryology of coelenterates and turtles; A. Agassiz, embryology of echinoderms and worms; H. J. Clark, embryology of turtles and systematic papers on sponges and coelenterates; E. Desor, echinoderms and embryology of worms; C. Girard, embryology, worms, and reptiles; J. Leidy, protozoa, coelenterates, worms, anatomy of mollusks; W. O. Ayres and T. Lyman, natural history of echinoderms; McCrady development of acalephs; W. Stimpson, marine invertebrates; A. E. Verrill, coelenterates, echinoderms, worms; A. Hyatt, evolutionary theories, bryozoa and mollusks; Pourtales, deep sea fauna; C. B. Adams, A. and W. G. Binney, Brooks, Carpenter, Conrad, Dall, Jay, Lea,

S. Smith, Tryon, mollusks; E. S. Morse, brachiopods, mollusks; J. D. Dana, coelenterates and crustacea; Kirtland, Loew, Edwards, Hagen, Melsheimer, Packard, Riley, Scudder, Walsh, insects; Gill, Holbrook, Storer, fishes; Cope, evolutionary theories, fishes and amphibia; Baird, reptiles and birds; J. A. Allen, amphibia, reptiles and birds; Brewer, Cassin, Coues, Lawrence, birds; Audubon, Bachman, Baird, Cope, Wilder, mammals.

The progress of ornithology in the United States previous to 1876 is well described in a paper by J. A. Allen in the American Naturalist (**10**, 536, 1876). A sketch of the early history of conchology is given by A. W. Tryon in the Journal (**33**, 13, 1862).

Jeffries Wyman was the most prominent comparative anatomist of this period. His work includes classic papers on the anatomy and embryology of fishes, amphibia, and reptiles.

The fifty volumes of the second series of this Journal, including the years 1846 to 1870, cover approximately this period of morphology and embryology. During this period the Journal occupied a very important place in zoological circles, for J. D. Dana was for most of this period the editor-in-chief, while Louis Agassiz and Asa Gray were connected with it as associate editors. Moreover, in 1864 one of the most promising of Agassiz's pupils, Addison E. Verrill, was called to Yale as professor of zoology and was made an associate editor in 1869.

In the Journal, therefore, may be found, in its original articles, together with its reports of meetings and addresses and its reviews of literature, a fairly complete account of the zoological activity of the period. The most important zoological researches, both in Europe and America, were reviewed in the bibliographic notices.

The most important series of zoological articles are by Dana himself. As his work on the zoophytes and crustacea of the U. S. Exploring Expedition continued, he published from time to time general summaries of his conclusions regarding the relationships of the various groups. Included among these papers are philosophical essays on general biological principles which must have had much influence on the biological studies of the time, and which form a basis for many of our present concepts.

The importance of these papers warrants the list being given in full. The titles are here in many cases abbreviated and the subjects consolidated.

General views on Classification, **1**, 286, 1846.
Zoophytes, **2**, 64, 187, 1846; **3**, 1, 160, 337, 1847.
Genus Astraea, **9**, 295, 1850.
Conspectus crustaceorum, **8**, 276, 424, 1849; **9**, 129, 1850; **11**, 268, 1851.
Genera of Gammaracea, **8**, 135, 1849; of Cyclopacea, **1**, 225, 1846.
Markings of Carapax of Crabs, **11**, 95, 1851.
Classification of Crustacea, **11**, 223, 425; **12**, 121, 238, 1851; **13**, 119; **14**, 297, 1852; **22**, 14, 1856.
Geographical distribution of Crustacea, **18**, 314, 1854; **19**, 6; **20**, 168, 349, 1855.
Alternation of Generations in Plants and Radiata, **10**, 341, 1850.
Parthenogenesis, **24**, 399, 1857.
On Species, **24**, 305, 1857.
Classification of Mammals, **35**, 65, 1863; **37**, 157, 1864.
Cephalization, **22**, 14, 1856; **36**, 1, 321, 440, 1863; **37**, 10, 157, 184, 1864; **41**, 163, 1866; **12**, 245, 1876.
Homologies of insectean and crustacean types, **36**, 233, 1863; **47**, 325, 1894.
Origin of life, **41**, 389, 1866.
Relations of death to life in nature, **34**, 316, 1862.

Of the above, the articles on cephalization as a fundamental principle in the development of the system of animal life have attracted much attention. The evidence from comparative anatomy, paleontology, and embryology alike supports the view that advance in the ontogenetic as well as in the phylogenetic stages is correlated with the unequal growth of the cephalic region as compared with the rest of the body. Dana shows that this principle holds good for all groups of animals. His homologies of the limbs of arthropods and vertebrates, however, do not accord with more modern views.

Other papers on the same and allied topics were published by Dana in other periodicals. His most conspicuous zoological works, however, are his reports on the Zoophytes and Crustacea of the United States Exploring Expedition, 1837-1842. The former consists of 741 quarto pages and 61 folio plates, describing over 200 new species, while the Crustacea report, in two volumes, has 1620 pages and 96 folio plates, with descriptions of about 500 new species. Each of these remains to-day as the most important contribution to the classification of the respective groups. The relationships of the species, genera and families were recognized with such remarkable judgment that Dana's admirable system of classification has remained the basis for all subsequent work.

Dana's critical reviews (**25**, 202, 321, 1858) of Agassiz's "Contribution to the Natural History of the United States" are among the most interesting of his philosophical discussions concerning the relationships of animals as revealed by their structure, their embryology, and their geological history.

The remaining zoological articles in this series cover nearly the whole range of systematic zoology. Especially important are the articles by Verrill on coelenterates, echinoderms, worms and other invertebrates.

In the years following the publication of Darwin's Origin of Species in 1859 occur many articles on the theory of natural selection. Some of the writers attack the theory, while others give it more or less enthusiastic support.

Experimental methods in solving biological problems were little used at this time, although a few articles of this nature appear in the Journal. Of these, a paper by W. C. Minor (**35**, 35, 1863) on natural and artificial fission in some annelids has considerable interest to-day.

Of the important zoological expeditions the following may be selected as showing their influence on American Zoology:

The North Pacific Expedition, with William Stimpson as zoologist, returned in 1856 with much new information concerning the marine life of the coasts of Alaska and Japan and many new species of invertebrates.

In 1867-1869 the United States Coast Survey extended its explorations to include the deep-sea marine life off the southeastern coasts and Gulf of Mexico under the leadership of Pourtales and Agassiz.

The Challenger explorations (1872-1876) added greatly to the knowledge of marine life off the American coast as well as in other parts of the world.

The explorations of the United States Fish Commission succeeded those of the Coast Survey in the collection of marine life off our coasts and in our fresh waters. These have continued since 1872 and have yielded most important results from both the scientific and economic standpoints.

Under the charge of Alexander Agassiz the Coast Survey Steamer "Blake," in 1877 to 1880, was engaged in dredging operations in three cruises to various parts of the Atlantic. The U. S. Fish Commission Steamer "Albatross," also in charge of Agassiz, made three expeditions in the tropical and other parts of the Pacific in the years from 1891 to 1905. The study of these collections has added greatly to our knowledge of systematic zoology and geographical distribution. The reports on some of the groups are still in course of preparation.

PERIOD OF EVOLUTION.

The time from 1870 to 1890 may be appropriately called the period of evolution, for although it commences eleven years after the publication of the Origin of Species, the importance of the natural selection theory was but slowly receiving general recognition. The hesitation in accepting this theory was due in no small degree to the opposition of Louis Agassiz. After the acceptance of evolution, although morphological and embryological studies continued as before, they were prosecuted with reference to their bearing on evolutionary problems.

Following closely the methods which had produced so much progress during the life of Agassiz, the field of zoology was now occupied by a new generation, among whom the pupils of Agassiz were the most prominent.

The teaching of biology at this time was also strongly influenced by Huxley, whose methods of conducting laboratory classes for elementary students were adopted in most of our large schools and colleges. This placed biology on the same plane with chemistry as a means for training in laboratory methods and discipline, with the added advantage that the subject of biology is much more intimately connected with the student's everyday life and affairs.

This increasing demand for instruction in biology and the consequent necessity for more teachers brought an increasing number of investigators into this field. New zoological text-books were also required. The "Standard Natural History," published in 1885, remains the most comprehensive general work on animals.

Conspicuous in this period was the work of E. D. Cope, best known as a paleontologist, but whose work on the classification of the various groups of vertebrates stands preeminent, and whose philosophical essays on evolution had much influence on the evolutionary thought of the time. He was a staunch supporter of the Lamarckian doctrine. Alpheus Hyatt also maintained this theory, and brought together a great accumulation of facts in its support. He thereby contributed largely to our knowledge of comparative anatomy and embryology. A. S. Packard, whose publications cover a wide range of topics, was best known for his text-books of zoology and his manuals on insects.

W. K. Brooks was a leading morphologist and embryologist. S. F. Baird, for many years the head of the United States Fish Commission, was the foremost authority on fish and fisheries and is also noted for his work on reptiles, birds and mammals. The man of greatest influence, although by no means the greatest investigator, was C. O. Whitman. It is to him that we owe the inception of the Marine Biological Laboratory, the most potent influence in American zoology to-day; the organization of the American Morphological Society, the forerunner of the present American Society of Zoologists; and the establishment of the Journal of Morphology. G. B. Goode was distinguished for his work on fishes and for his writings on the history of science.

E. L. Mark, C. S. Minot, and Alexander Agassiz were acknowledged leaders in their special fields of research—Mark in invertebrate morphology and embryology, and Minot in vertebrate embryology, while Alexander Agassiz made many important discoveries in the systematic zoology and embryology of marine animals, and to him we owe in large measure our knowledge of the life in the oceans of nearly all parts of the world.

The knowledge of the representatives of the different divisions of the American fauna had now become sufficient to allow the publication of monographs on the various classes, orders and families. At this time also particular attention was given to the marine invertebrates of all groups.

Of the many investigators working on the various groups of animals at this time only a few may be mentioned. The protozoa were studied by Leidy, Clark, Ryder, Stokes; the sponges by Clark, Hyatt; the coelenterates by A. Agassiz, S. F. Clarke, Verrill; the echinoderms by A. Agassiz, Brooks, Kingsley, Fewkes, Lyman, Verrill; the various groups of worms by Benedict, Eisen, Silliman, Verrill, Webster, Whitman; the mollusks by A. and W. G. Binney, Tryon, Conrad, Dall, Sanderson Smith, Stearns, Verrill; the Brachiopods by Dall and Morse; the Bryozoa by Hyatt; the crustacea by S. I. Smith, Harger, Hagen, Packard, Kingsley, Faxon, Herrick; the insects by Packard, Horn, Scudder, C. H. Fernald, Williston, Norton, Walsh, Fitch, J. B. Smith, Comstock, Howard, Riley and many others; spiders by Emerton, Marx, McCook; tunicates by Packard and Verrill; fishes by Baird, Bean, Cope, Gilbert, Gill, Goode, Jordan, Putnam; amphibians and reptiles by Cope; birds by Baird, Brewer, Coues, Elliott, Henshaw, Allen, Merriam, Brewster, Ridgway; and the mammals by Allen, Baird, Cope, Coues, Elliott, Merriam, Wilder.

Interest in the evolutionary theory continued to increase and eventually developed into the morphological and embryological studies which reached their culmination between 1885 and 1890 under the guidance of Whitman, Mark, Minot, Brooks, Kingsley, E. B. Wilson and other famous zoologists of the time. In these years the Journal of Morphology was established and the American Morphological Society was formed.

The morphological, embryological and paleontological evidences of evolution as indicated by homologies, developmental stages and adaptations were the most absorbing subjects of zoological research and discussion.

The third series of the Journal (1870-1895), likewise including fifty volumes, embraces this period of zoological activity in morphological and embryological studies, culminating with the inception of the modern experimental methods.

In this period also occurred the greatest progress in marine systematic zoology, due to the explorations of the United States Fish Commission off the Atlantic Coast. The Journal had an important share in the zoological development of this period also, for A. E. Verrill, who was now an associate editor, was in charge of the collections of marine invertebrates. Consequently most of the discoveries in this field were published in the Journal in numerous original contributions by Verrill and his associates. The explorations of the U. S. Fish Commission Steamer "Albatross" are described from year to year by Verrill, with descriptions of the new species of invertebrates discovered.

The numerous original contributions by Verrill on subjects of general zoological interest as well as on those of a systematic nature give this third series of the Journal much zoological importance. Verrill's papers cover almost the whole field of descriptive zoology, but are mainly devoted to marine invertebrates. Those which were originally contributed to the Journal or summarized by him in his literature reviews include the following topics:

Sponges, **16**, 406, 1878.

Coelenterates, **37**, 450, 1864; **44**, 125, 1867; **45**, 411, 186, **46**, 143, 1868; **47**, 282, 1869; **48**, 116, 419, 1869; **49**, 370, 1870; **3**, 187, 432, 1872; **6**, 68, 1873; **21**, 508, 1881; **6**, 493, 1898; **7**, 41, 143, 205, 375, 1899; **13**, 75, 1902.

Echinoderms, **44**, 125, 1867; **45**, 417, 1868; **49**, 93, 101, 1870; **2**, 430, 1871; **11**, 416, 1876; **49**, 127, 199, 1895; **28**, 59, 1909; **35**, 477, 1913; **37**, 483, 1914; **38**, 107, 1914; **39**, 684, 1915.

Worms, **50**, 223, 1870; **3**, 126, 1872.

Mollusks, **49**, 217, 1870; **50**, 405, 1870; **3**, 209, 281, 1872; **5**, 465, 1873; **7**, 136, 158, 1874; **9**, 123, 177, 1875; **10**, 213, 1875; **12**, 236, 1876; **14**, 425, 1877; **19**, 284, 1880; **20**, 250, 251, 1880; **2**, 74, 91, 1896; **3**, 51, 79, 162, 355, 1897.

Crustacea, **44**, 126, 1867; **48**, 244, 430, 1869; **25**, 119, 534, 1908.

Ascidians, **1**, 54, 93, 211, 288, 443, 1871; **20**, 251, 1880.

Dredging operations and marine fauna, **49**, 129, 1870; **2**, 357, 1871; **5**, 1, 98, 1873; **6**, 435, 1873; **7**, 38, 131, 405, 409, 498, 608, 1874; **9**, 411, 1875; **10**, 36, 196, 1875; **16**, 207, 371, 1878; **17**, 239, 258, 309, 472, 1879; **18**, 52, 468, 1879; **19**, 137, 187, **20**, 390, 1880; **22**, 292, 1881; **23**, 135, 216, 309, 406, 1882; **24**, 360, 477, 1882; **28**, 213, 378, 1884; **29**, 149, 1885.

Miscellaneous, **39**, 221, 1865; **41**, 249, 268, 1866; **44**, 126, 1867; **48**, 92, 1869; **3**, 386, 1872; **7**, 134, 1847; **10**, 364, 1875; **16**, 323, 1878; **20**, 251, 1880; **3**, 132, 135, 1897; **9**, 313, 1900; **12**, 88, 1901; **13**, 327, 1902; **14**, 72, 1902; **15**, 332, 1903; **24**, 179, 1907; **29**, 561, 1910.

S. I. Smith describes the metamorphosis of the crustacea (**3**, 401, 1872; **6**, 67, 1873) species of crustacea (**3**, 373, 1872; **7**, 601, 1874; **9**, 476, 1875), and dredging operations in Lake Superior (**2**, 373, 448, 1871). In this series occurs also a series of papers on comparative anatomy and embryology from the Chesapeake Zoological Laboratory in charge of W. K. Brooks. In the 39th and 40th volumes of the third series (1890) occur several papers on evolutionary topics by John T. Gulick (**39**, 21; **40**, 1, 437) which have attracted much attention.

Before the end of this period, however, this Journal was relieved from the necessity of publishing zoological articles by the establishment of several periodicals devoted especially to the various fields of zoology. We find, therefore, but few exclusively zoological papers after 1885, although articles of a general biological interest and the reviews of zoological books continue.

In the fourth series of the Journal, beginning in 1896, occur also a number of articles on systematic zoology by Verrill and others and several papers having a general biological interest. Brief reviews of a small number of zoological books are still continued, but at the present day the Journal, which played so important a part in the early development of American zoology, has been given over to the geological and physical sciences in harmony with the modern demand for specialization.

PERIOD OF EXPERIMENTAL BIOLOGY.

Zoological studies remained in large measure observational and comparative until about 1890 when the experimental methods of Roux, Driesch and others came into prominence. Interest then turned from the accumulation of facts to an analysis of the underlying principles of biological phenomena. The question now was not so much what the organism does as how it does what is observed, and this question could be answered only by the experimental control of the conditions. These experimental studies met with such remarkable success that in a few years the older morphological studies were largely abandoned, the Morphological Society changed its name to the Society of Zoologists, and in 1904 the Journal of Experimental Zoology was established. The experimental methods were applied to all branches of biological science, and while it must be freely admitted that little progress has been made toward an understanding of the ultimate causes which underlie biological phenomena, a great advance has been made in the elucidation of the general principles involved.

Experimental embryology, histology, regeneration, comparative physiology, neurology, cytology, and heredity have in recent years successfully adopted an experimental aspect and have made significant progress thereby. Biology has now taken its place beside chemistry and physics as an experimental science.

The latest great advance in biology has been in the field of heredity. The rediscovery of the Mendelian principles of heredity in 1900 brought to light the most important generalization in biology in recent times. The new science of genetics is essentially the experimental study of heredity.

We are at the moment in the midst of an effort to establish in biology a few relatively simple laws by using for the purpose the vast accumulations of observational data gathered in past years, supplemented by such experimental data as have been provided by these more recent investigations. Such hypotheses as have been formulated are for the most part only tentatively held, for their validity is generally incapable of a critical test. But wherever such tests have been possible, the laws of mathematics, physics and chemistry are found applicable to biological phenomena.

The number of investigators has now become so great and their activities so prolific that the list and synopses of the zoological publications each year cover upwards of 1000 to 1500 pages in the International Catalogue of Scientific Literature.

American Leadership.—During the first half of the century the progress of zoology in America remained distinctly behind that of Europe. At the beginning of the century the science was farthest developed by the French and English, although Linnæus was a Swede and took his degree in Holland. Under the influence of Von Baer and his monumental treatise on embryology (Ueber Entwicklungsgeschichte der Thiere, 1828), and supported later by the great physiologist, Johannes Müller, whose "Physiologie des Menschen" (1846) forms the basis of modern physiology, the German school forged rapidly ahead and eventually assumed the leadership in zoology, as in several other branches of science.

In the latter half of the century the influence of the German universities dominated in a large measure the zoological investigations in America. The reason for this is partly due to the fact that many of our young zoologists, after finishing their college course, completed their preparation for research by a year or more at a German university. The more mature zoologists, too, looked forward with keen anticipation to spending their summer vacations and sabbatical years in research in a German laboratory or at the famous Naples station in which the German influence was dominant.

With the rise of experimental biology since 1890, however, the American zoologists have shown so high a degree of originality in devising experiments, so much skill in performing them, and such keenness in analyzing the results, that they have assumed the world leadership in several of the special fields into which the science of zoology is now divided.

Biological Periodicals.

Perhaps in no better way can the progress of biology in America be illustrated than by a brief survey of the origin and development of the more important biological journals. For it will be seen that these publications have become more numerous and more specialized as the science has advanced in specialization.

The early publications—which as is well known, treated mainly of the birds, mammals and other vertebrates, and of insects, crustacea and shells—consisted mainly of separate books or pamphlets, published by private subscription. After the establishment of the so-called Academies of Science, or of Arts and Sciences, toward the end of the 18th and in the first quarter of the 19th century, the reports of the meetings began to be published as periodical Journals, supported by the academies. In these publications, and in this Journal which was founded at the same time, appear papers on all branches of science, including zoology. As soon as zoology in America assumed its modern aspects through the influence of Louis Agassiz and his followers the earliest strictly zoological journals were established.

It should be noted, however, that the journals of the scientific and natural history societies were more or less fully devoted to zoological topics according to the nature of the activities of the members and correspondents. After the establishment of the Museum of Comparative Zoology by Louis Agassiz came the founding in 1863 of its Bulletin and later its Memoirs. These publications have continued to the present day as a standard of excellence for the reports of zoological investigations. In connection with the systematic work on mollusks, the American Journal of Conchology was established in 1865. The American Naturalist was founded in 1867 by four of Louis Agassiz's pupils, Hyatt, Morse, Packard and Putnam. It was later edited by Cope as a leading periodical for the publication of biological papers, particularly those relating to evolution, and is at present devoted to evolutionary topics. It is now in the 52nd volume of its new series.

With the awakened interest in comparative anatomy and embryology came the need for an American journal which should supply a means of publication for the reports of researches accomplished by the increasing number of workers in these fields. This need was fully met by the establishment of the Journal of Morphology in 1887. This publication, now in its 30th volume, has equalled the best European journals in the character of its papers. A few years later (1891) came the Journal of Comparative Neurology for the publication of investigations relating to the morphology and physiology of the nervous system and to nervous and allied phenomena in all groups of organisms. Twenty-eight volumes of this journal have been completed. The Zoological Bulletin was started under the auspices of the Marine Biological Laboratory in 1897 for the publication of papers of a less extensive nature and which could be more promptly issued than those in the Journal of Morphology where elaborate plates were required. After two years the scope of the Bulletin was enlarged to include botanical and physiological subjects. The name was correspondingly changed to the Biological Bulletin. Of this important periodical 33 volumes have been issued.

For the publication of papers on human and comparative anatomy and embryology, the American Journal of Anatomy was established in 1901, and is now in its twenty-third volume.

Meanwhile the trend of zoological interest was toward topics connected with the ultimate nature of biological phenomena. The meaning of these phenomena could be determined only by the experimental method. Researches in this field became more prominent and the adequate publication of the numerous papers required the establishment of a new journal in 1904. This was named the

Journal of Experimental Zoology. It immediately took its place in the front rank of American zoological periodicals. Twenty-four volumes have been published.

In spite of the constantly increasing number of journals, the science grew faster than the means of publication. So crowded did the American journals become that long delays often resulted before the results of an investigation could be issued. This condition was met in part by the sending of many papers to be published in European journals (a necessity most discreditable to American zoology) and in part by the establishment ef additional means of publication. Of the latter the Anatomical Record, now in its fourteenth volume, was begun in 1906 for the prompt publication of briefer papers on vertebrate anatomy, embryology and histology and for preliminary reports and notes on technique.

During the past few years has come a great advance in the experimental breeding of plants and animals. Problems in heredity and evolution have taken on a new interest since the importance and validity of Mendel's discovery have been recognized. To meet this development of biology the journal Genetics was begun in 1916 for the publication of technical papers, while the Journal of Heredity, modified from the American Breeders Magazine, is devoted to popular articles on animal and plant breeding, and Eugenics.

On the whole, the science of zoology is now assuming a closer relation to practical affairs. Entomology, for example, is now represented by the Journal of Economic Entomology, of which 10 volumes have been issued since 1907. The Journal of Animal Behavior covers another practical field of research. The Proceedings of the Society for Experimental Biology and Medicine, starting in 1903, the American Journal of Physiology, and several other publications cover the physiological field. Among other important periodicals are the following:

The Journal of Parasitology, established 1914, now in its fourth volume, is devoted to the interests of medical zoology. The Auk, now in the 34th volume of its new series (42d of old series) is the official organ of the American Ornithologists Union and is devoted to the dissemination of knowledge concerning bird life. The Annals of the Entomological Society of America, established in 1908, and now in its 10th volume, is one of several important entomological journals. The Nautilus, of which 28 volumes have been issued, is one of the more successful journals devoted to conchology.

In addition to these are the many volumes of systematic papers in the Proceedings of the United States National Museum, the practical reports in the Bulletin of the United States Fish Commission, the vast literature issued yearly by the various divisions of the United States Department of Agriculture, Public Health Service and other Governmental departments, while the list of publications by scientific societies, museums, and other institutes is constantly increasing and covers all fields of biological research.

At the present time facilities for the publication of research on any branch of zoology are as a rule entirely adequate. For this highly satisfactory condition the science is indebted to the support given five of its most important journals by the Wistar Institute of Anatomy and Biology.

BIOLOGICAL ASSOCIATIONS.

An important light on the history of biology in America can be thrown by a glance at the rise and development of societies or associations for the report and discussion of papers relating to that branch of science. In the first half of the 19th century natural history societies were formed in most cities and centers of learning. These were very important factors in the promotion of scientific research as well as in the diffusion of popular knowledge of living things. The aims and activities of twenty-nine such scientific societies, many of which were devoted especially to natural history, are described in one of the early volumes of the Journal (10, 369, 1826). The Connecticut Academy of Arts and Sciences, dating from 1799, and the Philadelphia Academy of Natural Sciences from 1812 are among the oldest of those which still exist.

Of national institutions the American Philosophical Society was founded in 1743, the American Academy of Arts and Sciences in 1780, and the National Academy of Sciences in 1863.

The American Association for the Advancement of Science, with its thousands of members, now has separate sections for each of the special branches of science. This society, organized in 1850, is said to have been the successor of the Association of American Geologists and Naturalists. This was itself a revival of the American Geological Society which first met at Yale in 1819. Its meetings have given a great support to the scientific work of the country.

In 1890, toward the end of the period in which morphological studies were being emphasized, the professional zoologists of the eastern states founded the American Morphological Society. This association held annual meetings during the Christmas holidays for the presentation of zoological papers. This name became less appropriate after a few years because of the gradual decrease in the proportion of morphological investigations owing to the greater attention being directed to problems in experimental zoology and physiology. Consequently the name was changed to the American Society of Zoologists. To be eligible for membership in this society a person must be an active investigator in some branch of zoology, as indicated by the published results.

The American Society of Naturalists was founded in 1883. The original plan of the society was for the discussion of methods of investigation, administration and instruction in the natural sciences, but its program is now entirely devoted to discussions and papers of a broad biological interest. It also arranges for an annual dinner of the several biological societies and an address on some general biological topic.

The American Association of Anatomists includes in its membership investigators and teachers in comparative anatomy, embryology, and histology as well as in human anatomy. Many professional zoologists and experimental biologists present their papers before this society.

These national societies have been of great service in fostering a high standard of zoological research. A still more important service, though generally less conspicuous, is rendered by the journal clubs in connection with all the larger zoological laboratories, and by local scientific societies which are now maintained in all the larger centers of learning throughout the country. There are also specific societies for some of the different fields of biological work.

BIOLOGICAL STATIONS.

No insignificant factor in the development of biological science has been the establishment of biological stations where investigators, teachers and students meet in the Summer vacation for special studies, discussions and research. The most successful of these laboratories have been located on the seashore and here the study of marine life in Summer supplements the work of the school or university biological courses. The famous Naples Station was founded in 1870, and was shortly after followed by several others. Similar biological stations are now supported on almost every coast in Europe and in several inland localities.

The first such American school was established by Louis Agassiz at the island of Penikeese on the coast of Massachusetts in 1873, succeeding his private laboratory at Nahant. During that Summer more than forty students gained enthusiasm for the work of future years. Unfortunately the laboratory so auspiciously started was of brief duration, for the death of Agassiz occurred in December of the same year, and the laboratory was discontinued at the end of the following Summer. Shortly afterward Alexander Agassiz equipped a small private laboratory at Newport, Rhode Island, and W. K. Brooks established the Chesapeake Bay Zoological Laboratory.

At this time the United States Fish Commission was engaged under the direction of Spencer F. Baird in a survey of the marine life of the waters off the Eastern Coast. Between 1881 and 1886 the Commission established the splendidly equipped biological station at Woods Hole, Massachusetts. Both here and at the Fish Commission Laboratory at Beaufort, North Carolina, much work in general zoology as well as in economic problems is accomplished. These laboratories are designed particularly for specialists engaged in researches connected with the work of the Fish Commission.

A need was soon felt for a marine laboratory along broader lines, and one available to the students and teachers of the schools and colleges. To meet these requirements the Woods Hole Marine Biological Laboratory was started in 1887, as the successor to an earlier laboratory at Annisquam, and has since become a great Summer congress for biologists from all parts of the country. It is safe to say that no other institution has been of equal service in securing for biology the high plane it now occupies in American science. The leading spirit in the establishment of this laboratory and its director for many years was Charles O. Whitman.

Successful marine laboratories are located also at Cold Spring Harbor, Long Island; at Harpswell, Maine; and at Bermuda. The Carnegie Institution maintains a laboratory at Tortugas Island, Florida, for the investigation of tropical marine life.

On the Pacific Coast marine laboratories are located at Pacific Grove and at La Jolla, California, and at Friday Harbor, Washington. Several other biological laboratories are open each Summer on our coasts, as well as a number of fresh-water laboratories on the interior lakes. There are also several mountain laboratories. The influence of these laboratories on American biology is immeasurable.

NATURAL HISTORY MUSEUMS.

Museums of Natural History or "Cabinets of Natural Curios" as they were sometimes called, were established in the first half of the 19th century in connection with the various natural history societies. These were of much service in stimulating the collection of zoological "specimens" and in arousing a popular interest in natural history.

The zoological museum of earlier days consisted of rows on rows of systematically arranged specimens, each carefully labelled with scientific name, locality, date of collection and donor—much like the pages of a catalogue. All this has now been changed; the bottles of specimens have been relegated to the storeroom, and the great plate glass cases of the modern museum represent individual studies in the various fields of modern zoological research, or individual chapters in the latest biological text-books. Often the talent of the artist and the skill of the taxidermist are cunningly combined to produce most realistic bits of nature.

The United States National Museum, the American Museum of Natural History, the Field Columbian Museum and the Museum of Comparative Zoology are among the finest museums of the world, while many of the states, cities, and universities maintain public museums as a part of their educational systems.

SYSTEMATIC ZOOLOGY AND TAXONOMY.

The work in systematic zoology is now mainly carried on by specialists in relatively small groups of animals. This is necessitated both by the increasingly large number of species known to science and by the completeness and exactness with which species must now be defined. The majority of systematic workers are now connected with museums where the large collections furnish material for comparative studies.

Prominent in this field is the United States National Museum, the publications of which are mainly taxonomic and zoogeographic, and cover every group of organism. The adequacy of this great museum for such studies may be illustrated by the collection of mammals. This museum has the types of 1135 of the 2138 forms (including species and subspecies) of North American mammals recognized in Miller's list,[4] and less than 200 forms lack representatives among the 120,000 specimens of mammals. Systematic monographs of several of the orders of mammals have been published.

Systematic study of the birds has brought the number of species and subspecies known to inhabit North and Middle America to above 3000. The most comprehensive systematic treatise is the still incomplete report of Ridgeway[5] of which seven large volumes have already been issued.

On the reptiles, the most complete monograph is that by Cope[6] entitled "The Crocodilians, Lizards and Snakes of North America."

The Amphibia have also been studied by Cope, whose report on the Batrachia of North America[7] is the standard taxonomic work.

The most comprehensive systematic work on fishes is the Descriptive Catalogue of the Fishes of North and Middle America by Jordan and Evermann.[8]

The invertebrate groups have been in part similarly monographed by the members of the U. S. National Museum staff and others, and further studies are in progress. Other taxonomic monographs published by this museum include the various groups of animals from many different parts of the world.

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[4] List of North American Land Mammals in the United States National Museum, 1911. Bull. 79, U. S. Nat. Mus., 1912.

[5] Birds of North and Middle America, Bull. 50, parts I-VII, U. S. Nat. Mus., 1901-1916.

[6] Report U. S. Nat. Mus. for 1898, pp. 153-1270, 1900.

[7] Bull. 34, U. S. Nat. Mus., 1889.

[8] Bull. 47, parts I-IV, U. S. Nat. Mus., 1896-1900.

A number of the larger State, municipal, and university museums publish bulletins on special groups represented in their collections as well as articles of general zoological interest.

Expeditions, subsidized by museum and private funds, are from time to time sent to various parts of the world and their results are often published in sumptuous manner.

The total number of living species of animals is unknown, but considering that about a quarter of a million new species have been described during the past thirty years, it is probable that several million species are in existence to-day. More than half a million have been described. These are probably but a small fraction of the number that have existed in past geological ages.

Thus, in spite of all the work that has been done in systematic zoology and as the number of known species continues to increase, there still remain many groups of animals, some of which are by no means rare or minute, in which probably only a small proportion of the species are as yet capable of identification.

It is only since the publication of Ward and Whipple's "Fresh-water Biology" within the past year that the amateur zoologist could hope to find even the names of all the organisms to be found in a single pool of water.

During the past few years there has been a tendency on the part of some of our biologists engaged in experimental work to disparage the studies of the systematists. It must be granted, however, that both lines of work are essential to the sound development of zoological science, for experimental investigations in which the accurate diagnosis of species is ignored always result in confusion.

Ecology.—The marvelous modifications in structure and instincts by which the various animals are adapted to their surroundings now forms a special topic in biological research and one of the most fascinating. The adaptations in habitat, time, behavior, appearance and even in structure are found capable of a certain individual modification when studied experimentally.

Zoogeography.—Closely associated with systematic zoology, and indeed a part of the subject in its broader sense, is the study of the geographical distribution of animal species and larger groups.

Paleontology.—The geological succession of organisms embraces a field where zoologist and geologist meet. Most of the studies in this subject, however, have been made by geologists.

BIOMETRY.

Since Darwin's theory of evolution postulated the origin of new species by means of natural selection, it was obviously necessary in order to apply a critical test to determine the precise limits of a species. It was, therefore, proposed to subject a given species to a strict examination by the application of statistical methods to determine the range of variation of its members and the extent to which the species intergrades with others. Other problems, particularly those concerning heredity, were treated in similar manner. This branch of biological science was particularly developed by the English School, led by Sir Francis Galton, followed by Karl Pearson and William Bateson.

In America the methods of biometry have been utilized extensively by Charles B. Davenport, Raymond Pearl, H. S. Jennings and others in the solution of problems in genetics and evolution. Their work shows the great value of critical statistical analysis in the interpretation of biological data. A thorough training in mathematics is now found to be hardly less important for the biologist than is a knowledge of physics and chemistry, for the science of biometry has become one of the most important adjuncts to the study of genetics.

COMPARATIVE ANATOMY AND EMBRYOLOGY.

Comparative Anatomy.—Upon the foundations laid down by Cuvier a century ago the present elaborate structure of comparative anatomy of animals, both vertebrate and invertebrate, has been developed. Vast as is the present accumulation of facts and theories many important problems still await their solution. Jeffries Wyman was long a leader in this field, where many workers are now engaged.

Embryology.—The embryological studies, so brilliantly begun by Von Baer early in the nineteenth century, are still in progress. They have now been entended to the groups more difficult of investigation and into the earliest stages of fertilization and implantation in the mammals. Artificial cultural methods have yielded important results. Louis and Alexander Agassiz, Mark, Minot, Brooks, Whitman, Conklin and E. B. Wilson have taken prominent parts in this work.

In the early nineties embryological studies were directed to the arrangement of cells in the dividing egg, and there was much discussion of "cell lineage" in development. Valuable as were these studies they threw comparatively little light on the general problems of evolution.

Experimental Embryology.—A more fertile field, developed at the same period and a little later, was found in experimental embryology. The discoveries made by Driesch and others in shaking apart the cells of the dividing egg or by destroying one or more of these cells gave a new insight into the potency of cells for compensatory and regenerative processes. These studies attracted many able investigators, who made still further advance by subjecting the germ cells, developing eggs, embryos, and developing organs to a great variety of artificial conditions.

Artificial Parthenogenesis.—Another question concerns the nature of the process of fertilization and the agencies which cause the fertilized egg to develop into an embryo. In 1899 Jacques Loeb succeeded in causing development in unfertilized sea-urchin eggs by subjecting them to concentrated sea water for a period and then returning them to their normal environment. To this promising field of experimental work came many of the foremost biologists both in America and Europe. It was soon found that the eggs of most groups of animals except the higher vertebrates could be made to develop into more or less perfect embryos and larval forms by treatment with a great variety of chemical substances, by increased temperature, by mechanical stimuli and by other means. This artificial parthenogenesis, as it is called, has also been successful in plants (*Fucus*), and recently Loeb has reared several frogs to sexual maturity by merely puncturing with a sharp needle the eggs from which they were derived. Loeb, then, maintains that "the egg is the future embryo and animal; and that the spermatozoon, aside from its activating effect, only transmits Mendelian characters to the egg.'"[9]

Further experimental analyses of the nature of the fertilization mechanism have recently been made by Morgan, Conklin, F. R. Lillie, and others.

Germinal Localization.—The question as to whether the egg contains localized organ-forming substances has been studied experimentally particularly by means of the centrifuge. The results indicate that neither of the older opposing theories of "performation" or "epigenesis" is applicable to all eggs, but that in certain organisms the eggs possess a well-marked differentiation while in others each part of the egg is essentially, although probably not absolutely, equipotential.

The Germplasm Cycle.—Since Weismann's postulation of the independence of soma and germplasm in 1885 many attempts have been made to trace the path of the hereditary substance from one generation to the next. A recent book by Hegner[10] summarizes the success attained in various groups of animals.

CYTOLOGY.

Another important field of investigation which has attracted many workers is that which pertains to the life of the cell—the science of cytology. Although the cell-theory was established as early as 1839, little advance was made in this subject in America before 1880. Since that time, however, Americans have been so successful in cytological discoveries that they are now among the world's leaders in this field.

These studies have been followed along both descriptive and experimental lines. The most prominent of the early workers in this field are E. L. Mark and E. B. Wilson. Mark's description of the maturation, fecundation, and segmentation of the egg is the most accurate and complete of the early cytological studies. Wilson's discoveries concerning the details of fertilization and his "Atlas of Fertilization and Karyokinesis," published in 1895, have now become classic. Wilson, too, has published the only American text-book on cytology,[11] and has more recently taken the lead in studies concerning the relation between the chromosomes and sex. Besides Wilson, Montgomery, Mark, McClung, Morgan, Miss Stevens, Conklin and their associates and students have now furnished conclusive evidence that the sex of an organism is determined by, or associated with, the nuclear constitution of the fertilized egg. This constitution is moreover shown to be dependent upon the chromosomes received from the germ cells.

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[9] J. Loeb, The Organism as a Whole, p. 126, 1916.

[10] The Germ-cell Cycle in Animals, 1914.

[11] The Cell in Development and Inheritance, 1896; second edition, 1900.

This explanation is in strict accordance with the results of experimental breeding. It is also quite in harmony with the Mendelian law of inheritance, and in fact forms one of the strongest supports for the view that all Mendelian factors are resident in the chromosomes. Recent work has also discovered the mechanism which governs the complicated conditions of sex which occur in those animals which exhibit alternating sexual and parthenogenetic generations. These remarkable processes are in all cases found to depend upon a definite distribution of the chromosomes.

Other recent experimental work has shown that while the sex is thus normally determined in the fertilized egg, it is in some animals not irrevocably fixed, and the normal effect of the sex chromosomes may be inhibited by abnormal conditions in the developing embryo, as is demonstrated by the recent work of Lillie and others.

The cytological basis for Mendelian inheritance has been very extensively studied by Morgan and his pupils in connection with their work on inheritance in the common fruit fly *Drosophila*. The evidence supports Weismann's earlier hypothesis that the chromosomes are the bearers of the heritable factors, and that these are arranged in a series in the different chromosomes. This theory is shown to be in such strict accord with both the cytological studies and the results of experimental breeding that Morgan has ventured to indicate definite points in particular chromosomes as the loci of definite heritable factors, or genes.

Confirmation of this view is furnished by the behavior of the so-called sex-linked characters, the genes for which are situated in the same chromosome as that which carries the sex factor. Many ingenious breeding experiments indicate further that all the hereditary characters in *Drosophila* are borne in four great linkage groups corresponding with the four pairs of chromosomes which the cells of this fly possess.

COMPARATIVE PHYSIOLOGY.

None of the experimental fields has been of greater importance in zoological progress than that which concerns the functions of the various organs. Without this companion science morphology and comparative anatomy would have become unintelligible. American investigators, among whom G. H. Parker stands prominent, have taken a leading part in this field also.

Neurology.—The physiological analysis of the components of the nervous system, both in vertebrates and invertebrates, is another important branch of experimental biology. The 28 volumes of the Journal of Comparative Neurology attest the large influence that American investigators have had in the development of this science.

Regeneration.—Experimental studies on the powers of regeneration in plants and animals have been made from the earliest times. During the past few years, however, there has been made a concerted attempt to analyze the factors which determine the amount and rate of regeneration. Much progress has been made toward the postulation of definite laws applicable to the regenerative processes of the parts of each organism. The critical analyses of Child have been particularly stimulating.

Tissue Culture.—Another line of experimental work which has been developed within the past few years by Harrison, Carrell, and others is the culture of body tissues in artificial media. These experiments have included the cultivation in tubes or on glass slides of the various tissues of numerous species of animals. They have yielded much information regarding the structure, growth and multiplication of cells, the formation of tissues, and the healing of wounds.

Transplantation and Grafting.—Closely associated experiments consist in the transplantation of organs or other portions of the body to abnormal positions, to the bodies of other animals of the same species or of other species. In this way much has been learned about the potentiality of organs for self-differentiation, for regulation, for regeneration and for compensatory adaptations. The experiments have shown, further, the independence of soma and germplasm and have revealed the nature of certain organs whose functions were previously obscure.

Tropisms and Instincts.—Another field of experimental biology concerns the analysis of behavior of organisms in response to various forms of stimuli. These studies are being prosecuted on all groups of organisms, including the larval stages of many animals, and are yielding most remarkable results. The success in this field of research is largely due to stimulating influence of Jacques Loeb, Parker, Jennings, and their co-workers.

Biological Chemistry.—Still another experimental field which has developed into one of the most important of the biological sciences relates to the fundamental chemical and physical changes which underlie all organic phenomena. A knowledge of both physiological and physical chemistry is to-day essential for all advanced biological work. The peculiar nature of life itself, of growth, disease, old-age, degeneration, death and dissolution are presumably only manifestations of chemical and physical laws. The ultimate goal of all experimental biology, therefore, will be reached only when the basic physico-chemical properties of life are understood. At that time only will the perennial controversy between vitalism and mechanism be ended.

ECONOMIC ZOOLOGY.

A moment's reflection will show that economic biology is the most essential of all sciences to the human welfare and progress. For man's relation to his environment is such that the penalty for ignorance or neglect of the biological principles involved in the struggle for existence quickly overwhelms him with a horde of parasites or other enemies.

It is only by the intelligent application of biological knowledge that our food supplies, our forests, our domesticated animals and our bodies can be protected from the ever ravenous organisms which surround us.

The losses to food supplies and other products by insects alone amounts to 100 millions of dollars a month in the United States. And the parasites cause losses in sickness and premature deaths each year of many millions more. Then there are the destructive rodents and other animals which add largely to our burdens of support. These enemies next to wars and fungi are the most destructive agencies on earth. Could they but be eliminated man's struggle against opposing forces would be in large measure overcome. The results of recent work in economic zoology, both in regard to the destruction of enemies and protection of useful mammals, birds and fishes, furnish a bright outlook for the future.

Protozoology.—Partly as an experimental field for the solution of general biological problems and partly because of its practical applications the study of protozoa has now developed into a special science.

The results of the investigations of Calkins, Woodruff, Jennings and others have greatly supplemented our understanding of the signification of such important biological phenomena as reproduction, sexual differentiation, conjugation, tropisms, and metabolism.

From an economic standpoint the protozoa have recently been shown to be of the greatest importance because of the human and animal diseases for which they are responsible.

Parasitology.—The animal parasites of man, domesticated animals and plants include numerous species of protozoa, worms, and insects. Together with the bacteria and a few higher fungi they cause all communicable diseases. When we consider that not only our health but also our entire food supply is dependent upon the elimination of these organisms we must admit that parasitology is the most important economically of all the sciences.

The reports of the investigations of Stiles and his associates in the Hygienic Laboratory and of Ransom and his staff in the Bureau of Animal Industry are widely distributed by the federal government. The systematic studies so ably begun by Joseph Leidy in the middle of the last century have been continued by Ward, Linton, Pratt, Curtis and others on the parasites of many groups of animals.

Economic Entomology.—Another extremely important biological science, the practical applications of which are second only to those of parasitology in importance, is entomology. In the last few years economic entomology has exceeded any of the other branches of biology in the number of its investigators. The American Association of Economic Entomologists has a membership of about five hundred. The work of most of these is supported by appropriations from the State and federal governments, and the results of their investigations are widely published.

It is now well known that some of the protozoon parasites are conveyed from man to man only through the bites of insects. The local eradication of several of our most fatal diseases has recently been brought about by the application of measures to destroy such insects. This is the greatest triumph of economic zoology.

Economic Ornithology and Mammalogy.—In addition to the local bird clubs and the American Ornithologists Union for the study and preservation of bird and mammal life, the Bureau of Biological Survey has for some years conducted investigations on the economic importance of the various species. The publications of this Bureau are of great value both in determining the economic status of our birds and mammals, and also in recommending means for the protection of the beneficial species and the destruction of the injurious. Several of the States issue similar publications.

Economic Ichthyology.—The U. S. Fish Commission has for many years been actively engaged in investigations on the food fishes, including methods for increasing the food supply by suitable protection and artificial propagation. The work includes also edible and otherwise useful mollusks and crustacea. Their marine and fresh-water laboratories have also been of great service to general biological science.

GENETICS.

One of the most interesting chapters in biology relates to the development of the modern science of heredity, or genetics.

Previous to the year 1900, when the Mendelian principle of inheritance was re-discovered, the relative importance of heredity and of environment in the development of an organism was little understood. It is true that Weismann had insisted on the independence of soma and germplasm some years earlier (1883), but the body of the individual was still generally considered the key to its inheritance.

The recognition of the general application of Mendel's discovery gave a great impetus to experimental breeding both in plants and animals. While heretofore it had been necessary to depend upon the somatic characters as evidence of the hereditary constitution of an individual, it now became possible, knowing the hereditary constitution of the parents of any pair of individuals, to predict with almost mathematical certainty the characters of their possible offspring.

In general, the laws of possible chance combinations of any group of characters determine the probability of any particular offspring possessing one or many of those characters. The physical basis for such Mendelian inheritance is evidently the chance combinations of chromosomes which result from the processes of maturation and union of the germ cells.

Certain limitations to the law are met with because the relatively small number of chromosomes involves linkage of genes, because of the occasional interchange of groups of genes between homologous chromosomes, and because the relative activity or potency of any particular gene may differ in different races, and, finally, because the normal activity of any given gene may be modified or inhibited by the action of other genes. It is by no means certain, however, that all inheritance is Mendelian, for there still remains much evidence that the hereditary basis of certain characters may be resident in the cytoplasm, rather than in the chromosomes. A recent book by Morgan, Sturtevant, Müller and Bridges (1915), entitled "the mechanism of Mendelian heredity" gives the cytological explanation of Mendelian inheritance.

Americans have from the first taken a leading part in this field of research and have been quick to recognize its practical applications to the improvement of breeds in both animals and plants. This prominent position is largely due to the experimental work of Castle, Davenport, Morgan, Jennings, Pearl, and their co-workers on animals and that of East, Emerson, Davis, Hayes and Shull on plants.

The geneticist now realizes that the appearance of the body (phenotype) gives but little clue to the inheritance (genotype). That two white flowers produce only purple offspring, or two white fowls only deeply colored chickens, or that a pair of guinea pigs, one of which is black and the other white, have only gray agouti offspring, while other apparently similar white flowers or white animals produce offspring like themselves, is now readily comprehensible and mathematically predictable.

The most important application of our newly acquired knowledge of inheritance is in the improvement of the human race. The wonderful opportunity in this direction must be apparent to all. The welfare of humanity depends upon the immediate adoption of eugenic principles. The Eugenics Record Office has secured many of the essential data.

With the destruction of the world's best germ plasm at a rate never equalled before, the outlook for the future race would be appalling were it not for the hope that with the advent of a righteous peace will come a realization of the necessity of applying these new biological discoveries to improving the races of men. That the discoveries have been made too late in the world's history to be of such use to humanity must not be thought possible.

EVOLUTION.

Previous to the publication of Darwin's Origin of Species in 1859, American zoologists were generally inclined toward special creation, in spite of the evidences for evolution which had been presented by Erasmus Darwin, Lamarck, and Geoffroy St. Hilaire. Indeed this attitude of mind continued for some years after the publication of the natural selection theory of Darwin and Wallace. This was in part due to the powerful influence of Louis Agassiz who bitterly opposed the Darwinian theory. As late as 1876, J. D. Dana only half-heartedly accepted it as indicated in his last essay on "Cephalization a fundamental principle in the development of animal life" (**12**, 245-251, 1876). He says:

> "The method by repeated creations through communications of Divine power to nature should be subordinated, as much as any other, to molecular law and all laws of growth; for molecular law is the profoundest expression of the Divine will, the very essence of nature; and no department of nature is without its appointed law of development. But the present state of science favors the view of 'progress through the derivation of species from species, with few occasions for Divine intervention. For the development of Man, gifted with high reason and will, and thus made a power above Nature, there was required, as Wallace has urged, the special art of a Being above Nature, whose supreme will is not only the source of natural law, but the working force of Nature herself,' and this I still hold." He further explains that cephalization "is not at all at variance with

Darwinism.'' In his later years he gave the theory his complete, though reluctant, acceptance.

A modified Lamarckian doctrine was widely accepted in the last quarter of the century, due largely to the influence of Cope, Hyatt and Packard. The inheritance of ''acquired characters'' demanded by this theory seems incompatible with the discoveries of recent times, so that ''today the theory has few followers amongst trained investigators, but it still has a popular vogue that is widespread and vociferous.''[12]

The origin of new varieties and species by accidental modifications of the germplasm is now the most widely accepted theory of evolution.

Some of the most important discoveries regarding the origin of new forms have been recently made by Morgan and his pupils. From a stock of the common fruit fly (*Drosophila ampelophila*) more than 125 new types have arisen within six years. Each of these types breeds true. ''Each has arisen independently and suddenly. Every part of the body has been affected by one or another of these mutations.'' To arrange these mutations arbitrarily into graded series would give the impression of an evolutionary series, but this is directly contrary to the known facts concerning their origin, for each mutation ''originated independently from the wild type.'' ''Evolution has taken place by the incorporation into the race of those mutations that are beneficial to the life and reproduction of the individual.'' This evolutionary process is usually accompanied by the elimination of those forms which have remained stable or which have developed adverse mutations.

A question that is being vigorously debated at this time concerns the possible effects of selection on the hereditary factors. Are the genes fixed both qualitatively and quantitatively or does a given gene vary in potency under different conditions and in different individuals? In the former case selection can only separate the existing genes into separate pure strains. But if the gene be quantitatively variable, then selection will result in the establishment of new types.

Castle has long stoutly maintained the effect of such selection, and his forces have recently been augmented by Jennings. The experimental work now in process will doubtless yield a decisive answer.

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[12] Morgan, T. H. A critique of the theory of evolution, p. 32, 1916.

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CONTRIBUTION TO THE STUDY OF EPITHELIAL MOVEMENT. THE CORNEAL EPITHELIUM OF THE FROG IN TISSUE CULTURE

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The mechanism of the movement of epithelium, embryonic as well as adult, has been discussed by various observers, but still further investigation is needed by the use of the tissue-culture method, where direct observation is possible.

The experimental work reported in the present paper is concerned with a study of the movement of corneal epithelium, and especially with its behavior with reference to certain mechanical supports.

Several papers have already appeared which deal with the movement of epithelium of various kinds in vitro (Ruth, '11, Champy, '14, Loeb, '02, Osowski, '14, and others). The observation of frog skin in vitro has been described by Holmes ('14) and Uhlenhuth ('14) recently in detail. Previous to this, Harrison ('10) Carrel and Burrows ('11), Lambert and Hanes ('13), and others mentioned it briefly in their papers on tissue culture. Among the works which deal with the culture of the corneal epithelium, that of Oppel ('12), who made use of the cornea of certain warm-blooded animals in his investigations, demands special attention. Harde ('16) made brief mention of an active lateral spreading of the corneal epithelium in the culture of vaccinia with corneal tissue. However, the materials which were used by Oppel and Harde are evidently not very suitable for direct observation. For this purpose cornea of more simple structure is desirable. It must be added that a number of investigations on the problem of the wound healing of the cornea have been made, such as those of Peters ('85), Salzer ('11), Löwenstein ('13), and others, which must, of course, be taken into consideration.

I. BEHAVIOR OF EPITHELIUM CULTIVATED IN VITRO

The cornea of the adult frog (especially R. pipiens) was used. After thoroughly washing its whole surface with sterilized Ringer's solution by means of a pipette, the entire cornea was cut out with a razor and put into Ringer's solution (or serum), after which it was divided into small pieces with very sharp scissors so that the fragments showed sharp edges. Pieces cut radially were preferred.

The cultures were all made by the hanging-drop method (Harrison, '10), the technique of which need not be detailed here. The piece of cornea was taken from the Ringer's solution and dropped on the surface of the cover-glass; excess solution was removed and a drop of plasma (or serum) run over the fragment; autoplasma was used in most of the cultures. The cover-glass was then inverted upon a thin glass ring and sealed on with vaselin (Harrison, '14).

Though all precautions were taken to keep the cultures free from bacteria, it was sometimes necessary to throw away a whole series as a result of infection. This was due obviously to the difficulty of perfect sterilization of the tissue.

In the aggregate, more than 1800 cultures were made, and in nearly all of the experiments two kinds of culture medium (plasma and serum) were used. The following descriptions are the results of the study of about 1500 cultures which were free from faulty technique.

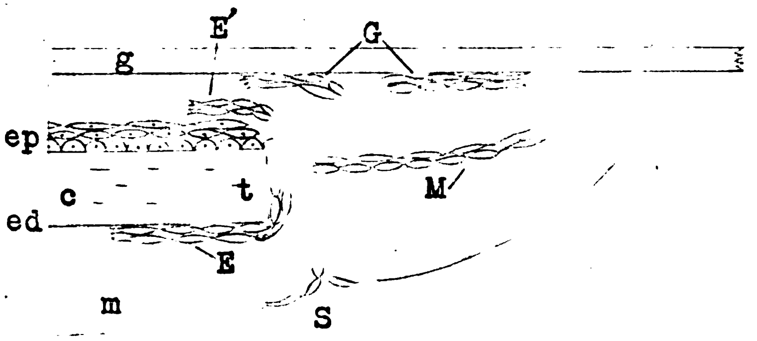

Fig. 1. Diagram showing various types of movement of corneal epithelium cultivated in plasma. *g*, cover-glass; *c*, tissue of cornea cultivated; *ep*, corneal epithelium; *ed*, endothelial surface; *m*, culture medium; *t*, cut end of the piece of cornea. Cell movement into the plasma (*M*), along the endothelial surface (*E*), on the epithelial surface (*E'*), along the cover-glass (*G*), and along the lower surface of plasma (*S*).

1. *Description of the types of epithelial movement in plasma (fig. 1)*

The cornea of the frog was very suitable for this purpose. For the observance of the intimate cell structure and a closer study of the mechanism of cell movement, high powers could be used. It was, of course, necessary to supplement the study of the living tissue by fixed and stained preparations of whole cultures and by serial sections. The observation of the living cultures was limited to the first week.

a. Movement in the medium. When a culture of cornea, freshly prepared in vitro, was examined under the microscope, the edges of the piece were seen to be sharply defined. One or two hours later the epithelium on the cut ends gave the impression of becoming a little translucent and swollen, a narrow clear rim appearing along the edges. Here and there, around the edges of the fragment, isolated round epithelial cells were to be seen, singly or in groups, having been detached by mechanical injury during the operation.

There was a short latent period before any movement was noticed, the first cellular activity appearing between the third and tenth hours. Accordingly, examination after twelve to twenty-four hours showed an active outgrowth of epithelial cells presenting amoeboid processes. As a rule, the corneal epithelium showed characteristic sheet-like extension during the period of active movement; the advancing edge was always furnished with an amoeboid border of hyaline ectoplasma, as has been described by Harrison ('10), Carrel and Burrows ('11), Lambert and Hanes ('13) and Holmes ('14). Some of the epithelium exhibited marked motility, recalling the movements of amoeba, so that an exact camera-lucida drawing could not be made; pseudopodia were formed, and through their activity the cells changed shape or moved from place to place. Some of the cells which showed filiform processes were over 0.2 mm. in length.

The strong tendency of the cells to lateral spreading brought about the formation of a continuous membrane, extending nearly horizontally, usually slanting upward a little toward the end. The spreading membrane also changed its direction of movement, showing contraction under various circumstances. Growth in strands was also observed. The growing epithelium, usually two cells in thickness, sometimes covered an area a little larger than the original corneal piece, whereby the cells became flattened and the intercellular spaces grew wider.

There was apparently little growth after the third (sometimes the fourth) day. The tissue itself then gave the impression of being less compact and more translucent than formerly. The epithelial cells showed a tendency to round off. The rupture of strands or sheets of cells into isolated masses was of frequent occurrence. The number of round cells increased rapidly from day to day, and generally fat droplets grew in number and size with the age of the culture, until the cells were often literally packed with them. No marked increase of mitotic figures was seen.

The activity of cell movement into the plasma depends on the consistency of the latter. Around the explanted tissue, liquefaction and retraction of the plasma were often observed which caused changes of the arrangement of cells.

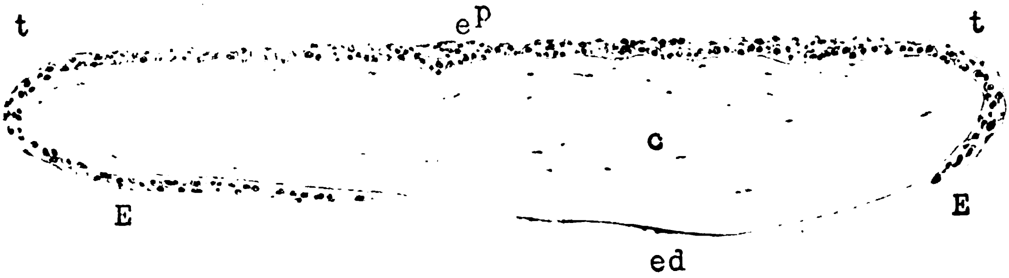

Fig. 2 Vertical section of corneal tissue cultivated in plasma. Experiment XXXVIII, 5, showing the epithelial movement (*E*) along the endothelial surface (*ed*). Age of the culture, four days. Drawn from one of the serial sections. *t*, cut ends; *ep*, epithelial, *ed*, endothelial surface; *c*, part of connective tissue of cornea. × 98.

b. Movement on tissue. The next important type of the epithelial movement is that on the corneal tissue. By virtue of this, the growing epithelium spreads over the edges of the fragment and along the endothelial surface. Figure 2 shows an example of this very clearly. A movement of this type occurred in some cases on all edges of a fragment and in others only on a part of it, combined with other types. It was also observed that part of the cut end of the epithelium might remain almost inactive, while in the other part marked activity occurred.

c. Other types of movement. Whenever the growing epithelial cells came into contact with the cover-glass, they moved actively over it. The character of cell movement, however, was essentially the same as on the endothelial surface. The advancing edge of the growth was composed of a very delicate sheet of protoplasm, showing amoeboid movement, with numerous branching hyaline pseudopodia. When the delicate membrane spread out to an extreme degree, it resulted in the breaking up of the sheet into isolated masses.

In a small percentage of the cultures the spreading of cells over the outer epithelial surface of the cornea (fig. 1, *E'*) as well as along the lower surface of the culture medium (fig. 1, S) was noted.

The elements of the connective tissue showed neither active growth nor movement. The study of the lymphocytes found in the corneal tissue needs special investigation.

2. Movement of the epithelium cultivated in serum

Generally, the cornea cultivated in serum showed some details of cell movement, different from those noted in plasma cultures. No amoeboid migration of the cells into the medium took place. During the first and second hours, the cells of the edges became clear and round, sometimes even swollen in appearance; they soon started to move.

The most striking and constant phenomenon noted in the use of serum was the spreading out of epithelium over the tissue itself, especially on the endothelial surface. The epithelial rim extended usually parallel with the cut edges, showing amoeboid movement on the advancing border. The rapidity of spreading out of cells was sometimes quite remarkable. In a small percentage of the cultures, movement of cells was also observed on the original outer epithelial surface of the cornea.

If single cells or a part of the rim were in contact with the cover-glass, they clung to it and spread over the surface of the glass with marked activity. The boundaries of individual cells were often hard to distinguish under the microscope. When such preparations are properly impregnated with silver nitrate (Lewis and Lewis, '12) the intercellular spaces can be clearly demonstrated (fig. 3). When the delicate membrane had extended to the utmost degree, the cells along the periphery became loose and isolated.

The appended tables give some examples of the frequency of various types of epithelial movement in plasma (table 1) and in serum (table 2), respectively.

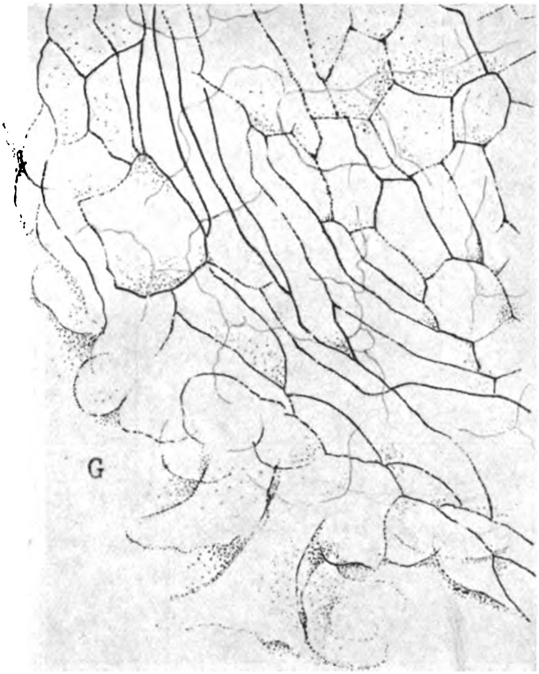

Fig. 3 Experiment XLIX, 2. Silver impregnation, demonstrating the boundaries of individual cells. Drawn from a part of actively moving border closely attached to the lower surface of cover-glass. Cultivated in serum; three days' growth. *G*, advancing border. × 450.

3. Velocity of epithelial movement

The velocity of cell movement in vitro exhibits a considerable variation under different circumstances, and, as stated before,

TABLE 1

Cultures in plasma

SERIES NO.	NUMBER OF PREPARATIONS	CELL MOVEMENT					NO MOVEMENT
		Into medium	On endothelial surface	On epithelial surface	On cover glass	On surface film	
50 a	18	14	14	1	1		
50 b	14	5	13				1
46	10	3	10		4		
45	6	5	5	2	2	1	1
43	20	13	11		2	1 (?)	
52	6	5	4	1	3		
57	10	9	3	2	4		
61	10	8	6	2	2	1	
62	2	2	2	2	2		
63	34	24	33				
102	33	18	31				
106	15	15	14				
104	15	1	15				
107	11	6	11				
110	8	6	8				
111	9	4	9				
212	18	11	17				
Total.....	239	149	206	10	20	3	2

combinations of several types of movement are often to be seen in a single preparation.

During the period of activity, a change in the type or direction of movement was often observed, and such a change in one part was apt to modify the movement of neighboring cells. Single cells or small groups of cells, as a rule, moved more freely than those in the spreading membrane. The consistency of the plasma, the liquefaction and the contraction of the fibrin influence both the rapidity and the type of movement into the plasma. The temperature has both direct and indirect influences on the movement. There is also individual variation which cannot be attributed to any of the above factors. For these reasons, the velocity of the epithelial movement varies from hour to hour, and it is very difficult to analyze the factors influencing it.

Usually, the most vigorous activity occurred during the first twenty-four hours. For instance, the epithelium on a piece of

TABLE 2

Cultures in serum

SERIES NO.	NUMBER OF PREPARATIONS	CELL MOVEMENT					NO MOVEMENT
		Into medium	On endothelial surface	On epithelial surface	On cover glass	On surface film	
56	28		28				
94	28		28		5	1	
58	7		6				1
60	8		7				1
139	7		7				
138	7		6				1
140	5		5				
135	8		8				
165	14		14				
166	12		12				
167	15		15				
208	23		23	1			
219	18		18				
221	20		20				
232	7		7				
233	9		9				
234	9		9				
Total.....	225		212	1	5	1	3

cornea cut radially into eight parts, spread out into the plasma within twenty-four hours in an extension which was even larger than their original area, though not in the same degree of rapidity at all cut ends. Similarly, in successful preparations, it was seen that the whole endothelial surface was covered within twenty-four hours by the epithelium spreading over the entire circumference of the piece. When the moving borders met each other, movement ceased.

Some measurements are given in table 3.

TABLE 3

	PREPARATION NO.	MOVEMENT ON ENDOTHELIAL SURFACE			MOVEMENT INTO PLASMA		
		After 5 hrs.	24 hrs.	48 hrs.	5 hrs.	24 hrs.	48 hrs.
Experiment 210, plasma culture, at 21°C.	1	0.14	0.4	0.5	0.06	0.3	0.5
	2	0.14	0.7	1.0	0.1	0.3	1.8
	3	0.1	0.8	0.8	0.11	1.1	1.6
	4	0.14	0.65	0.7	?	1.0	0.9
	5	0.14	1.0	1.0	0.08	0.8	1.0
	6	0.1	0.3	0.9			
	7	0.1	0.61	0.9			
	8	0.1	0.6	0.8			
Average movement in millimeters		0.12	0.63	0.83	0.087	0.7	1.16
					20 hrs.	40 hrs.	60 hrs.
Experiment 211A, plasma culture, at 20°C.	1				0.5	2.2	2.0
	2				0.8	4.0	5.4
	3				1.0	2.5	2.8
Average movement in millimeters					0.77	2.8	3.4
		20 hrs.	40 hrs.		20 hrs.		
Experiment 211B, plasma culture, at 20°C.	1	0.7	1.2				
	2	0.6	0.7				
	3	1.0	1.0				
	4	0.6	0.7				
	5	0.4	0.5		0.2		
	6	0.6	0.6				
Average movement in millimeters		0.65	0.78				
		20 hrs.					
Experiment 208, serum culture, at 20°C.*	1	0.3					
	2	0.4					
	3	0.8					
	4	0.8					
	5	0.6					
	6	0.7					
Average movement in millimeters		0.6					

*Pieces which measured: 1.2 x 0.6 mm.; 1.8 x 0.5 mm., 2.0 x 0.6 mm., 2.4 x 1.1 mm., and 0.8 x 0.8 mm., were entirely covered up with moving epithelium in seventeen hours.

II. ON THE REACTION OF EPITHELIAL CELLS TO CERTAIN SOLID SUPPORTS

1. *Movement on flat surfaces*

a. On glass and celloidin. We shall first consider movement on the glass cover-slip.[1] There is difficulty by means of usual culture method in bringing the epithelial rim into contact with the cover-glass from the beginning. Attempts to do this by reducing the plasma failed, as the epithelium did not show activity unless the medium was used in sufficient quantities.

In the later experiments, the piece of cornea (cut off tangentially to eyeball) was placed on the cover-slip, mainly with the inner surface down, and subjected to slight pressure by means of thin silver wires or glasses and kept in that position for a certain period after mounting with serum, so that the cut ends came into contact with the glass. When the silver wires were properly placed and controlled under the microscope, it was possible in almost every instance to bring certain parts of the moving epithelium into contact with the cover. Of course, where even a minimal space existed between the tissue and cover-glass surface, the epithelium crept on the underlying tissue.

Cover-slips coated with celloidin were also used. Thus the movement of the epithelium on celloidin and glass surfaces, respectively, could be compared. A comparison could also be made in the same preparation, by employing a cover-slip, only one half of which was coated with celloidin. Such preparations must be handled carefully, otherwise a change of cell form readily occurs.

No characteristic difference whatever in the mode of movement on the two different surfaces could be noticed. If the epithelium grew out vigorously, closely attached to the cover-slip, each cell became flattened into an exceedingly thin layer. In some instances it was seen that the advancing border became very irregular. This was most marked when active cells on the border, showing unusual protoplasmic activities, tore themselves loose from their connection. Similar conditions, however, were often to be noticed in the actively mobile epithelial layer growing out into firmly clotted plasma. In the epithelial movement on the endothelial surface, such a condition but rarely occurred; as a rule, the cells moved more uniformly, showing a smooth advancing border.

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[1] It should be stated here that the cover-glasses used in these experiments were throughly cleaned and washed in the vapor of distilled water, as is usually done to remove any trace of alkali, in order to exclude any chemical influence from that source. (Ostwald-Ruther, Physiko-chemische Untersuchungen.)

In other instances small pieces of cover-glass or celloidin membrane were placed on the endothelial surface and pressed against it, so that a certain portion or the whole of this surface, which is a favorable support for moving cells, was covered. In these cases the epithelium was also seen to move over the celloidin or glass, if they were suitably mounted and the cells were not injured. Where the cell movement on such artificial supports failed, examination proved that the placing of the membrane was not suitable; thus, by control of pressure, it was possible to direct the moving cells to these artificial surfaces, though this was not always an easy matter. At any rate, it is an established fact that the epithelium is able to creep on objects of such nature that chemical influences are excluded.

b. On dead cornea. If in a part of an explanted piece of cornea an epithelial defect existed, it was seen that the epithelial cells were able to cover the spot, spreading out from all edges, practically with the same rapidity as on the endothelial surface.

The same was true of a spot which had been killed by touching it with a heated needle. The cells injured by the latter procedure became round and detached, and the growing epithelium crept beneath them along the surface of the killed tissue.

Preparations were made of large strips of corneal tissue (about 2 x 4 mm.), across the center of which a sharply defined epithelial defect was produced, after which one half of the remaining corneal tissue was killed by means of a heated needle, making the tissue surface look opaque and wrinkled. In such preparations it was seen that the epithelial movement over the wound occurred uniformly, covering both the live and the dead tissue with the same rapidity.

Analogous experiments were made with pieces of cornea in which an epithelial defect existed at one extreme end and in which the tissue at the other end had been killed; similarly, the cell movement on a burnt wound was compared with a control preparation, in which the underlying tissue was left intact with simply an epithelial defect.

The use of tissue, vitally stained by neutral red[2] or nile blue, facilitated the observation of epithelial movement on such wound surfaces.

Analogous observations were made by using the surface of the cartilaginous layer of the sclerotic coat of the frog's eye. This tissue could readily be isolated from the other layers after boiling, and it was then thoroughly washed before using. It was translucent, showing characteristic convexity, which admirably fitted on the inner surface of the similarly curved cornea; even without any pressing, the two tissues would lie so closely together in the culture medium (serum) that the epithelium from the cut ends of cornea crept over the cartilage. It proved helpful, however, if the pieces were slightly pressed together.

Out of twenty-six experiments, movement of the epithelium over the cartilaginous plate took place in twenty-one; in three cases no movement was observed owing to injury of cells. An example of this is illustrated in figures. 4 and 5.

2. Movement of fiber-like supports

Next, the response of the epithelium to the various fiber-like supports were tested, such as spider web, silk fiber, glass wool, and asbestos.

a. Spider web as support. Experiments were made similar to those of Harrison ('14), using spider webs. In each preparation a sufficient quantity of serum was used as culture medium.

Figure 6 represents one of the preparations of the series; many cultures were found where the cells clung to the fibers. Out of thirty cultures twenty-four were positive, four doubtful, two infected.

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[2] S. Matsumoto. Demonstration of epithelial movement by the use of vital staining. This paper will appear in the next number of this journal.

b. Silk fiber as support. Other experiments were made, using silk fibers. In these a number of fibers of raw silk were stretched on the glass ring and cover-glass, imitating the experiment with the spider webs.

Out of twenty-nine cases there were fourteen that gave positive results, one being infected.

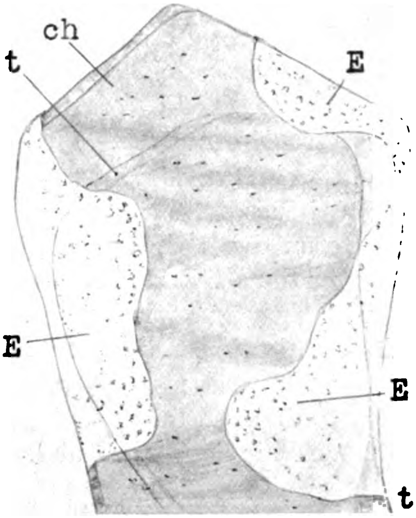


Fig. 4 Experiment 233,-4, showing epithelial movement (*E*) on the boiled cartilaginous plate (*ch*) of sclerotic coat closely placed on the endothelial surface of cornea. Cultivated in serum; forty hours' growth. *t*, cut ends of the piece of cornea are clearly visible through the transparent cartilagineous plate. × 98.

Similar experiments were made by Carrel and Burrows ('11a) with embryonic chick tissue.

c. Glass wool as support. Glass wool (Merck) was thoroughly cleaned and sterilized, then placed in the culture, so that the epithelium might attach itself to the fibers.

Out of twenty-eight cultures sixteen gave positive results. An example is shown in figure 7.

d. Asbestos fiber as support. Asbestos fibers were ignited and treated with pure conc. HCl; the fine cloudy suspension was collected and washed in distilled water to remove completely all traces of HCl, and then sterilized before use.

In the serum culture of the cornea the fibers were mixed densely so that, they appeared like a nest, in which the tissue lay. As the fibers were very fine, they did not hinder microscopical examination, if not too densely placed.

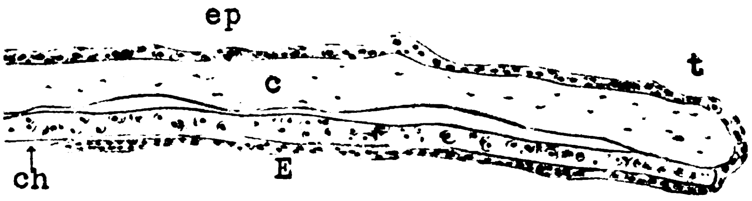


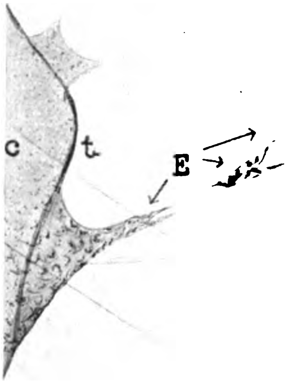
Fig. 5 Experiment 233; 4. Same preparation as shown in fig. 4. Portion of vertical section, drawn from one of the serial sections. *ep*, epithelial surface of cornea; *c*, connective tissue; *ch*, cartilaginous plate, placed on the endothelial surface of cornea; *t*, cut end of cornea. Note the epithelial movement (*E*) on the sclerotic cartilage (*ch*). Forty hours' growth. × 98.

Thirty cultures were made in this group, with positive results in twenty-two. Figure 8 shows an example of this series.

e. Movement on pith, etc. Further studies were made with pith. Thin pieces were used, which were kept in distilled water which was changed every three days for a period of over three months.

Experiments showed that the epithelium was able to cling to the cell walls of the thin piece of pith. Figure 9 shows clearly the growing out of epithelial cells on the support. Out of twenty-seven experiments nineteen gave positive results.

On the shell membrane of hen's egg, used as support, after thoroughly washing and boiling, a similar condition was observed.



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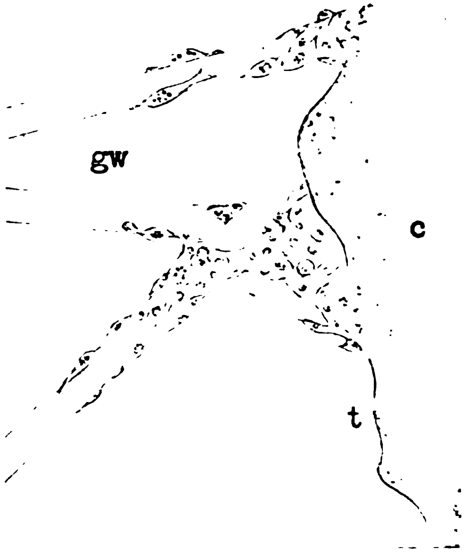

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Fig. 6 Experiment 238, 11. Epithelial movement in serum on spider web. In the neighborhood of the explanted tissue (*c*) a good many isolated cells, round in shape, were noticed, which are omitted in this figure; they showed no active movement. *t*, edge of the piece; above, some cells move out in sheets on the cover-glass. Compare Jour. Exp. Zoöl., 17, 521, figs. 4 to 7, 12. × 98.

Fig. 7 Experiment 49, 7. Epithelial movement on glass wool (*gw*); cultivated four days in serum. *t*, edge of tissue. × 98.

III. RÉSUMÉ

This paper deals with the movement of corneal epithelium of the adult frog in vitro. Frog cornea is very suitable for this purpose, as it is so transparent and thin that it permits of direct observation of the cell movements.

The corneal epithelium cultivated in plasma shows various types of movement, according to the nature of the substratum (fig. 1). The movement is of an amoeboid character. As a rule the cells have a strong tendency to cling to their own kind and thus extend in sheets, although under certain conditions active movement of isolated cells is also to be seen.

In the majority of cultures movement into the medium or along the endothelial surface (or both) takes place according to the consistency of the culture medium. The fact, that the epithelium moves along the epithelial or endothelial surface is very important from various points of view; such a movement may easily be overlooked in the culture of non-transparent tissue, such as skin.

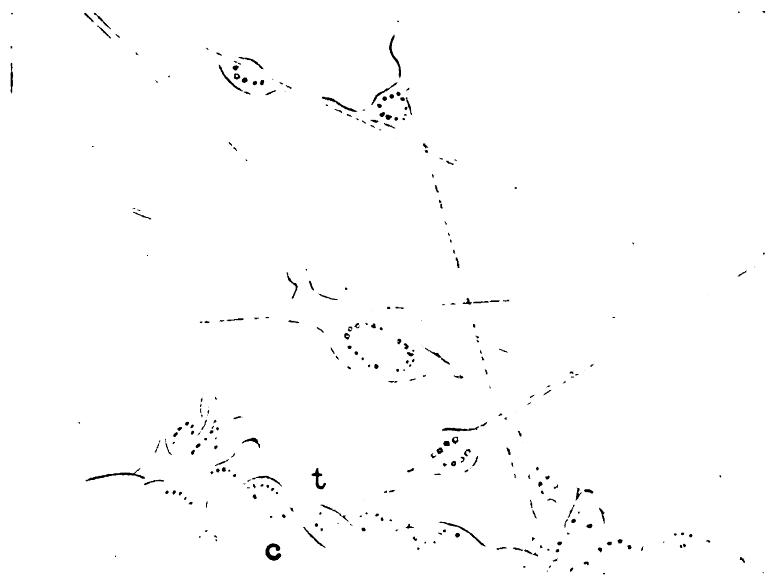

Fig. 8 Experiment 238, 1. Epithelial movement on fibers of asbestos: cultivated forty hours in serum. At the extreme left cells are to be seen moving along cover-slip. Note the adaptation of single cells to asbestos fibers. × 450.

In the preparations in which serum is used, no migration of the epithelium into the medium takes place. There is mainly a movement of cells over the tissue, especially on the endothelial surface.

Naturally, the question arises, whether the epithelial movement on the tissue, which is so frequently to be seen in the fluid medium, is chemotactic or thigmotactic in nature, It might even be that the type of movement is due both to mechanical and to chemical influences acting simultaneously. However, the epithelium is able to move with practically the same velocity both on a substratum where the covering epithelium has been simply scraped off and on one where the underlying tissue has been killed by heating. The movement in the latter cases cannot, therefore, be dependent upon chemotactic influences from the living tissue. The same is true of the movement taking place on the surface of the cartilaginous plate of the sclerotic coat, previously killed by boiling.

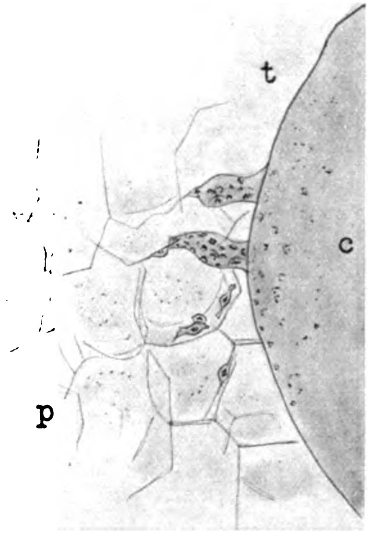

Fig. 9. Experiment 239. Corneal piece cultivated in serum with pith; three days after explanation. Note the epithelium clinging to the piece of pith (*p*) moving out of the cut end (*t*) of cornea (*c*). × 98.

Furthermore, the epithelium is able to move on the surface of glass, on a celloidin film, and also on such fiber-like supports as spider web, glass wool, asbestos, etc., when it is brought into contact with them.

The cell movement on the glass and celloidin film is very vigorous, sometimes more rapid than on the endothelial surface. In the movement on such supports chemotactic influences are to be considered as excluded.

It has been repeatedly observed by various writers that a suitable support for the growing cells is an important requisite; Harrison ('14) demonstrated lately the importance of such factors for the movement of embryonic cells very clearly. The facts above described confirm this view, that stereotropism plays an important rôle in cell movement.

The behavior of corneal epithelium in vitro serves to throw some light on the mechanism of epithelial growth in vivo.

The experiments show clearly that the epithelium is able to extend from the cut end quite rapidly in sheets into the medium (plasma), or on the tissue (plasma and serum), and can cover a large area, whereas mitotic cell divisions are not necessary at all.

That Oppel ('12) as well as Osowski ('14) did not observe amoeboid activity of the epithelium was perhaps due to the difficulty of direct observation. Special, careful observation on the movement of epithelium of warm-blooded animals, however, is necessary.

In conclusion, I wish to express my indebtedness to Prof. R. G. Harrison for his direction and valuable advice.

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BAKER ON THE MICROSCOPE AND THE POLYPE

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YALE UNIVERSITY

THE transactions of learned societies during the eighteenth century are replete with microscopical observations instigated directly or indirectly by the pioneer work of Hooke and Grew in England, Leeuwenhoek and Swammerdam in Holland, and Malpighi in Italy. In particular, Leeuwenhoek's long series of letters, published year after year chiefly in the *Philosophical Transactions* of the Royal Society of London, revealed an undreamed-of microcosm beyond the ken of unaided vision and turned the attention of the "Ingenious and Curious," the philosopher and dilettante, to the slowly developing microscope as a source of pleasure or fame. Among the English disciples of Leeuwenhoek, it was Henry Baker, of London, on whom seems to have fallen the Dutch microscopist's mantle—though, it is true, considerably reduced.

Baker was born in London on May 8, 1698, and began his career at an early age as a bookseller's apprentice. In 1720 he undertook to tutor a deaf and dumb child and with such success that he established a private school in London for deaf mutes. His course of instruction comprised speech and lip reading, writing and drawing, but the essential point of his system, which unfortunately he felt constrained to keep a secret, was that, after the preliminary training, he took his pupils on rambles about London and instructed them by conversation on the events of everyday life with which they came in contact. Baker realized a considerable fortune from his school, and the success of his method brought him to the attention of Daniel Defoe, who in 1728 became associated with him in founding the *Universal Spectator and Weekly Journal.* The following year Baker married Defoe's youngest daughter, Sophia, who bore him two sons. He survived both his wife and children, his sole heir being a grandson. After Baker's death on November 25, 1774, the Royal Society established the Bakerian Lecture with a fund given by him for discourses on "anatomical or chymical" subjects.

Baker's first essay as an author was "The Invocation of

Health," which was published without his sanction in 1723. The same year appeared a pamphlet of "Original Poems: Serious and Humorous," printed for the author, and was followed in three years by the "Second Part of Original Poems: Serious and Humorous." In 1727 was published his philosophical verses entitled "The Universe. A Poem intended to restrain the Pride of Man." This was printed for T. Worrall at Judge Coke's Head, against St. Dunstan's Church, in Fleetstreet, and sold for one shilling. "The Universe," as its subtitle indicates, is an attempt to impress the reader with the vastness and grandeur of nature and the folly of insignificant man believing that it was created for his especial edification. This poem, which attained considerable popularity and was frequently quoted, reminds one of the poetical works of Erasmus Darwin. An idea of the whole may be gathered from the following extract, which is one of the most interesting, and apparently one which Baker especially approved since he quoted it before the Royal Society—the first instance of original versification appearing in the *Philosophical Transactions*—and again nearly twenty years later in "The Microscope Made Easy":

> Each Seed includes a Plant: that Plant, again,
> Has other Seeds, which other Plants contain:
> Those other Plants have all their Seeds; and Those,
> More Plants, again, successively inclose.
>
> Thus, ev'ry single Berry that we find,
> Has, really, in itself whole Forests of its Kind,
> Empire and Wealth one Acorn may dispense,
> By Fleets to sail a thousand Ages hence:
> Each Myrtle-Seed includes a thousand Groves,
> Where future Bards may warble forth their Loves.
> So ADAM'S Loins contain'd his large Posterity,
> All People that have been, and all that e'er shall be.
>
> Amazing Thought! what Mortal can conceive
> Such wond'rous Smallness!—Yet we must believe
> What Reason tells: for Reason's piercing Eye
> Discerns those Truths our Senses can't descry.

During the next decade Baker published two volumes entitled "Medulla Poetarum Romanorum" and also a translation of Molière; but his name has been preserved from oblivion almost solely by his two volumes, "The Microscope Made Easy" and "Employment for the Microscope," which exploited the compound microscope.

For Many have been frightened from the Use of it, by imagining it required great Skill in Optics, and Abundance of other Learning to comprehend

> it to any Purpose: whereas nothing is really needful but good Glasses, good Eyes, a little Practice, and a common Understanding, to distinguish what is seen; and a Love of Truth, to give a faithful Account thereof. Others have considered it as a meer Play-thing, a Matter of Amusement and Fancy only, that raises our Wonder for a Moment, but is of no farther Service: which Mistake they have fallen into, from being unacquainted with any Principles whereby to form a right Judgment of what they see. Many, again, have laid the Microscope aside, after a little Use, for want of knowing what Objects to examine, where to find, how to prepare, and in what Manner to apply them. The trouble of managing it has also frighted some. . . .
>
> The likeliest Method of discovering Truth, is, by the Experiments of Many upon the same Subject; and the most probable Way of engaging People in Such Experiments, is, by rendering them easy, intelligible, and pleasant. To effect this, is my Endeavor in the following Treatise. . . .[1]

"The Microscope Made Easy" was dedicated to the Royal Society and, receiving its imprimatur in 1742, was published by the famous London bookseller, Robert Dodsley.

> Where Tully's bust and honour'd name
> Point out the venal page,
> There Dodsley consecrates to fame
> The classics of his age.[2]

The book's popularity is attested by the fact that a second edition, with some additions, appeared the following year, and later editions, unchanged, in 1744, 1754, 1769, 1785, etc., not to mention the translations into foreign languages. The scope of the work is given *in extenso* on the title page (*cf.* Fig. 1).

The spirit of the eighteenth century which called forth Baker's volumes is well expressed in the Introduction:

> In this inquisitive Age, when the Desire of Knowledge has spread itself far and wide, and we sit not down contented, as heretofore, with the Opinions of ancient Times, but resolve to examine for Ourselves, and judge from our own Experience; it may not, perhaps, prove unacceptable to point out some proper Subjects of Enquiry.
>
> The Works of Nature are the only Source of true Knowledge, and the Study of them the most noble Employment of the Mind of Man. Every Part of the Creation demands his Attention, and proclaims the Power and Wisdom of its Almighty Author. The smallest Seed, the minutest Insect, shews the Skill of Providence in the Aptness of its Contrivance for the Purposes it is to serve, and displays an Elegance of Beauty beyond the utmost Stretch of Art.
>
> The Wise in all Ages have been sensible of this Truth; and, as far as they were able, have studied and enquired into the Recesses of Nature; but for Want of proper Helps have frequently been mistaken. As certain Principles must first be learned ere we can become Masters of any Science, so in the School of Nature, we must begin with the *Minutiæ*, the smallest and most uncompounded Parts, ere we can understand the larger and more considerable. . . .

---

[1] "The Microscope Made Easy," Dedication.

[2] *London newspaper*, 1756.

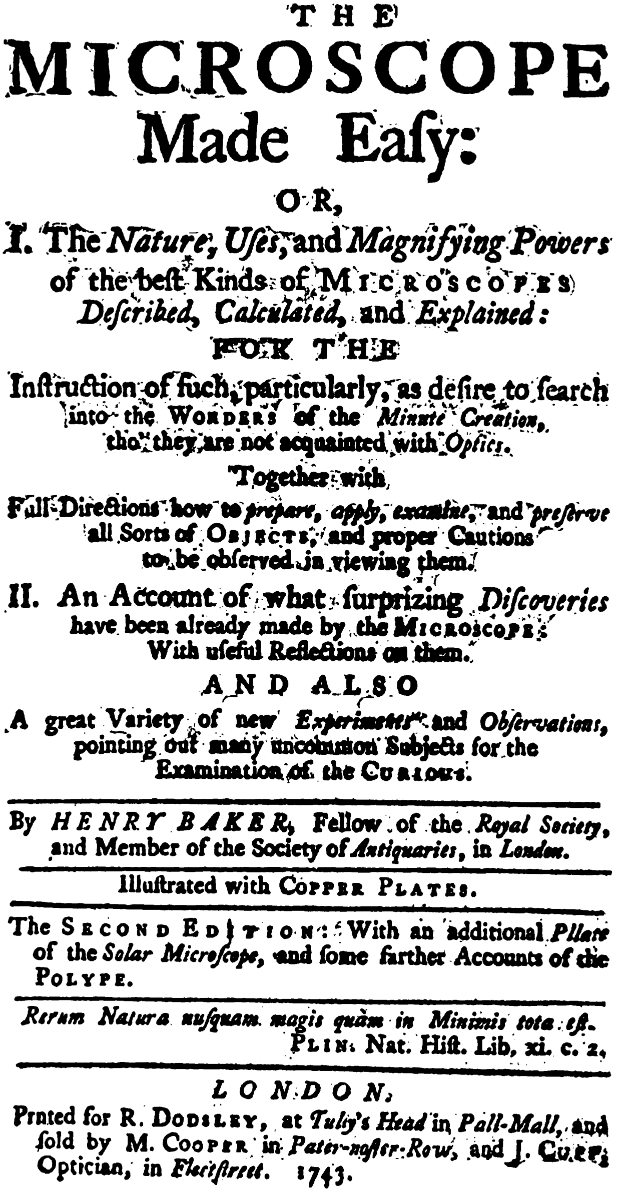
> That Man is certainly the happiest, who is able to find out the greatest Number of reasonable and useful Amusements, easily attainable and within his Power: and, if so, he that is delighted with the Works of Nature, and makes them his Study, must undoubtedly be happy; since every Animal, Flower, Fruit, or Insect, nay, almost every Particle of Matter, affords him an Entertainment. Such a Man never can feel his Time hang heavy on his Hands, or be weary of himself, for want of knowing how to employ his Thoughts: each Garden or Field is to him a Cabinet of Curiosities, every one of which he longs to examine fully; and he considers the whole Universe as a Magazine of Wonders, which infinite Ages are scarce sufficient to contemplate and admire enough. . . .
>
> All these, and numberless Wonders more, the MICROSCOPE can exhibit to us. I shall therefore proceed to describe this noble Invention, shew how far it is improved at present, give a brief Account of what Discoveries have been made, and point out some Objects for the Curious to examine by it. In doing this, I shall avoid as much as possible all Affectation of Learning, or Expressions that are not in common Speech, being desirous that every body may understand me.

The author, then, in Part I. of the book, first describes and illustrates in succession the Single Microscope and a new invention for "giving Light to it by a Speculum," the Double Reflecting Microscope, the Solar or Camera Obscura Microscope and the Microscope for Opake Objects, and then devotes the remaining chapters to general microscopical technique, concluding with some sound advice:

> Beware of determining and declaring your Opinion suddenly on any Object; for Imagination often gets the Start of Judgement, and makes People believe they see Things, which better Observations will convince them could not possibly be seen: therefore assert nothing till after repeated Experiments and Examinations, in all Lights, and in all Positions.
>
> When you employ the Microscope, shake off all Prejudice, nor harbour any favourite Opinions; for, if you do, it is not unlikely Fancy will betray you into Error, and make you think you see what you would wish to see.
>
> Remember, that Truth alone is the Matter you are in Search after; and if you have been mistaken, let no Vanity seduce you to persist in your Mistake.

Part II. of the work (*cf.* Fig. 1) is devoted to an account of microscopical discoveries and to "Pointing out many uncommon Subjects to the Enquiry of the Curious." The material presented is largely gleaned from the works of others, chiefly Leeuwenhoek, Swammerdam, Hooke, Power and Derham—to whose works he gives page references in every instance—but in addition he inserts many observations of his own, some new and some from his papers before the Royal Society, and also intersperses the whole with pertinent remarks in regard to the subject under immediate consideration. For example, in his account of blood:

> I believe it will be allowed, that where one Person dies from a Dis-



THE
MICROSCOPE
Made Eaſy:
OR,
I. The *Nature, Uſes,* and *Magnifying Powers*
of the beſt Kinds of MICROSCOPES
Deſcribed, Calculated, and *Explained:*
FOR THE
Inſtruction of ſuch, particularly, as deſire to ſearch
into the WONDERS of the *Minute Creation,*
tho' they are not acquainted with *Opticks.*
Together with
Full Directions how to *prepare, apply, examine,* and *preſerve*
all Sorts of OBJECTS, and proper Cautions
to be obſerved in viewing them.
II. An Account of what ſurprizing *Diſcoveries*
have been already made by the MICROSCOPE:
With uſeful Reflections on them.
AND ALSO
A great Variety of new *Experiments* and *Obſervations,*
pointing out many uncommon Subjects for the
Examination of the CURIOUS.

By *HENRY BAKER,* Fellow of the *Royal Society,*
and Member of the Society of *Antiquaries,* in *London.*

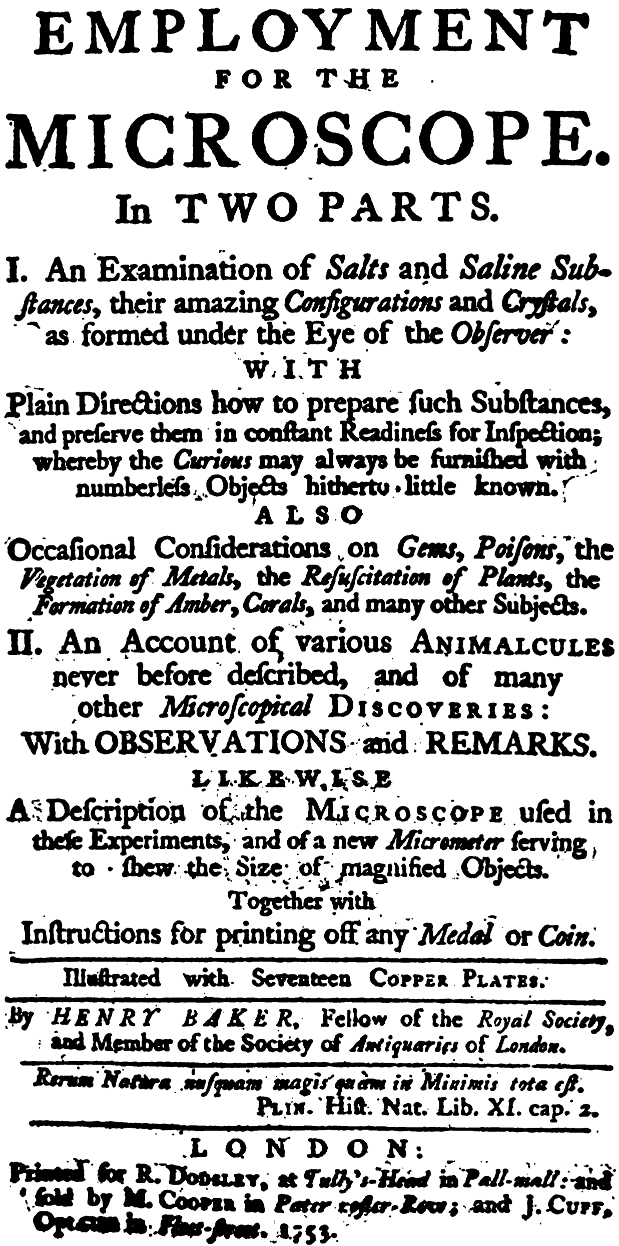
Illuſtrated with COPPER PLATES.

The SECOND EDITION: With an additional *Plate*
of the *Solar Microſcope,* and ſome farther Accounts of the
POLYPE.

Rerum Natura nuſquam magis quàm in Minimis tota eſt.
PLIN. Nat. Hiſt. Lib. xi. c. 2.

LONDON:
Printed for R. DODSLEY, at *Tully's Head* in *Pall-Mall,* and
ſold by M. COOPER in *Pater-noſter-Row,* and J. CUFF,
Optician, in *Fleetſtreet.* 1743.

FIG. 1.



EMPLOYMENT
FOR THE
MICROSCOPE.
In TWO PARTS.

I. An Examination of *Salts* and *Saline Subſtances*, their amazing *Configurations* and *Cryſtals*, as formed under the Eye of the *Obſerver*:
WITH
Plain Directions how to prepare ſuch Subſtances, and preſerve them in conſtant Readineſs for Inſpection; whereby the *Curious* may always be furniſhed with numberleſs Objects hitherto little known.
ALSO
Occaſional Conſiderations on *Gems*, *Poiſons*, the *Vegetation of Metals*, the *Reſuſcitation of Plants*, the *Formation of Amber*, *Corals*, and many other Subjects.

II. An Account of various ANIMALCULES never before deſcribed, and of many other *Microſcopical* DISCOVERIES:
With OBSERVATIONS and REMARKS.
LIKEWISE
A Deſcription of the MICROSCOPE uſed in theſe Experiments, and of a new *Micrometer* ſerving to ſhew the Size of magnified Objects.
Together with
Inſtructions for printing off any *Medal* or *Coin*.

Illuſtrated with Seventeen COPPER PLATES.

By *HENRY BAKER*, Fellow of the *Royal Society*, and Member of the Society of *Antiquaries* of *London*.

Rerum Natura nuſquam magis quam in Minimis tota eſt.
PLIN. Hiſt. Nat. Lib. XI. cap. 2.

LONDON:
Printed for R. DODSLEY, at *Tully's-Head* in *Pall-mall*: and ſold by M. COOPER in *Pater noſter-Row*; and J. CUFF, Optician in *Fleet-ſtreet*. 1753.

FIG. 2.

> order in the containing Vessels, twenty miscarry by some unnatural Alteration in the Fluids that pass through them: and therefore, if we can find what their natural State is, the Means whereby it may be preserved in such State, by what Accidents it may be prejudiced, and how it may be restored, our Pains will be well employed.
>
> In order to obtain this useful Knowledge, it will be necessary to examine the human Blood and other Juices, frequently, with the *Microscope*, in every Condition, and under every Distemper, as well as in a State of Health: by which we shall have ocular Demonstration of its different Appearances in each State, and of the Changes it undergoes; and by Experiments of various Mixtures with it, may possibly discover by what Means it can be altered from one Condition to another. . . .
>
> Would our learned Physicians, who are best able to judge of such Matter, be induced to take this Method into their Practice, it is reasonable to believe, that in a few Years the Causes of Diseases would be better known, and the Art of Healing brought to a much greater Certainty, than it is at present. . . .
>
> Many Distempers might perhaps be cured by an immediate Admission of some Medicine into the Veins, which elude the Power of all that can be taken by the Mouth. For the Stomach, by its Heat, its Action, and a Mixture of its Juices, works such an Alteration in Things before they can be admitted into the Blood, that they are unable to produce the same Effects as if they were received into it simply and unchanged.

In the chapter on the generation of organisms he says that

> Nothing seems now more contrary to Reason, than that *Chance* and *Nastiness* should give a Being to Uniformity, Regularity, and Beauty: that two such unlikely Principles should produce, in different Places, Millions of Vegetables of the same Kinds, and alike exactly, even in the most minute Particularities: or, what is yet more amazing, that dead *corrupting Matter*, and blind *uncertain Chance*, should create living Animals. . . . This, however, was the Opinion, not only of the Ignorant and Illiterate, but of the most learned grave Philosophers of preceding Ages; and would probably still have been taught and believed, had not *Microscopes* discovered the Manner how all these Things are generated. . . .

And, again, that

> The Growth of Animals and Vegetables seems to be nothing else but a gradual Unfolding and Expansion of their Vessels, by a slow and progressive Insinuation of Fluids adapted to their Diameters; until, being stretched to the utmost Bounds appointed them by Providence at their Formation, they attain their State of Perfection, or, in other Words, arrive at their full Growth

—a view which called forth the verses from *The Universe* which were quoted on an earlier page.

The numerous illustrations which are grouped on 15 copper plates include, for example, good reproductions of Leeuwenhoek's original figure of *Hydra* from the *Philosophical Transactions*, and what is probably the first figure of a *Paramecium*, from an anonymous communication in the same publication.

Concordant with the custom of the time:

> Before this Treatise is concluded, it will not perhaps be thought unprofitable to examine some of the finest and most exquisite Performances of human Art, and compare them with the Productions of Nature; as such a Comparison must tend towards humbling the Self-conceit and Pride of Man, by giving him a more reasonable and modest Opinion of himself; and at the same time may in some Degree conduce towards improving his imperfect Conceptions of the SUPREME CREATOR. . . .
>
> The Use of the *Microscope* will naturally lead a thinking Mind to a Consideration of *Matter*, as fashioned into different Figures and Sizes, whether Animate or Inanimate: it will raise our Reflections from a Mite to a Whale, from a Grain of Sand to the Globe whereon we live; thence to the Sun and Planets; and, perhaps, onwards still to the fixt Stars and the revolving Orbs they enlighten, where we shall be lost amongst Suns and Worlds in the Immensity and Magnificence of Nature.

Although the proportion of Baker's own observations is relatively very small, the work is far from being merely an excellent compilation, as it is permeated throughout with the spirit of a man who is a constant, enthusiastic devotee of microscopic study and who is acquainted first hand with a large part of the material he is presenting.

The success of "The Microscope Made Easy" led Baker to publish ten years later his "Employment for the Microscope," which is practically a supplement to the second part of his first book—and after 1753 the two works were usually sold together under the title "Baker on the Microscope." This second volume is largely a compilation of Baker's own studies of animalcules from various sources and here we find a Suctorian figured for the first time, apparently *Podophrya quadripartita*, as well as a number of other hitherto undescribed organisms. Baker, like Joblot, makes an attempt to give more or less appropriate names to the various organisms which he describes but he does not apply a binomial nomenclature or classify them—Dr. John Hill, during the previous year, being the first to give animalcules definite Latin names (*e. g.*, *Paramecium*) and to arrange them in groups, under the influence of the rapid advances in taxonomy at the time.

Although Baker's studies on microscopic organisms have been emphasized above, apparently he himself and the Royal Society were more pleased with his studies, presented at length in this volume, which he had been making

> for above ten Years past, on a great variety of Saline Bodies, Mineral, Vegetable and Animal, as well as many other Substances, both simple and compound, whose Parts can be dissolved in Fluids, after a Method which has never hitherto been described by any Author, or practised before myself by any body that I have heard of. And tho' I have found their original Particles undiscoverable by any Microscope, the Time I hope has not been wholly misemployed; since I have been enabled, by the help of that

> Instrument, to behold the amazing Order and Regularity, wherewith, after being separated by Dissolution, they come together and re-unite under the Eye, when put in Action by certain Degrees of Heat, in Configurations appropriated to each of them respectively, and with a Constancy that is surprising.[3]
>
> The Experiments here described, and which the Reader is instructed to make, must I think generally entertain; but merely to entertain, is, I hope, the least of their Worth. They may possibly lead to the Knowledge of what passes in the Formation of Gems, and the most beautiful mineral Productions: And as every new Discovery is an Encouragement to farther Disquisition, the Hints here given may perhaps set abler Heads at Work to improve Art on the Principles of Nature. Examinations by the Microscope, in the Manner here directed, may likewise be employed to ascertain the Truth and Purity of many simple Substances and Compositions made use of in Medicine, and detect Fraud and Imposition.[4]

For this work Baker received the Copley Medal of the Royal Society for 1774 which was awarded "to whomsoever of the members shall be deemed to have produced the most extraordinary Discovery during the whole year."

The volume concludes with a chapter on "Miscellaneous Observations" which includes a section on the microscopes of Leeuwenhoek.

> Though Mr. Leeuwenhoek's Microscopes are much talked of, very few People are acquainted with their Structure and Apparatus, no Figure of them that I remember having ever been made publick: 'tis therefore hoped the Curious will be pleased to see a Drawing of them, taken with great Exactness from those in the Repository of the *Royal Society*. . . .

Baker was apparently a congenial spirit and "Societarian," being for nearly thirty years one of the most active Fellows and frequent members of the Council of the Royal Society, a Fellow of the Antiquarian Society of London, and a founder and secretary of the Society for the Encouragement of Arts, Manufactures and Commerce. Martin Folkes, during his eleven-year presidency of the Royal Society, was on terms of considerable intimacy with Baker, being one of a small group of the Fellows who met now and then at Baker's home to consider matters philosophical. Folkes apparently had great confidence in Baker's skill with the microscope and frequently asked him to verify observations communicated to the Royal Society through the president.

It was in this capacity, for example, that Baker made his observations on the origin of "Eels in blighted wheat" (which Needham had communicated in his "New Microscopical Discoveries," London, 1745, and later advanced as support of the

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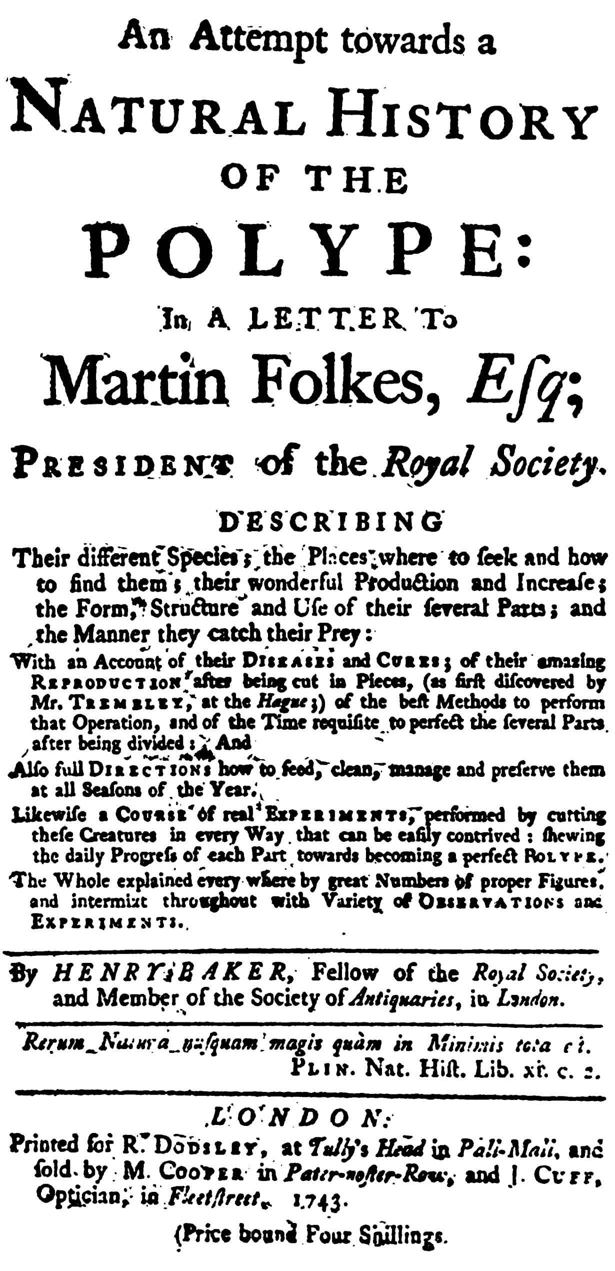
[3] "Employment for the Microscope," Chapter I.

[4] *Ibid.*, Dedication.



An Attempt towards a

NATURAL HISTORY

OF THE

POLYPE:

In A LETTER To

Martin Folkes, *Eſq*;

PRESIDENT of the *Royal Society*.

DESCRIBING

Their different Species; the Places where to ſeek and how to find them; their wonderful Production and Increaſe; the Form, Structure and Uſe of their ſeveral Parts; and the Manner they catch their Prey:

With an Account of their DISEASES and CURES; of their amazing REPRODUCTION after being cut in Pieces, (as firſt diſcovered by Mr. TREMBLEY, at the *Hague*;) of the beſt Methods to perform that Operation, and of the Time requiſite to perfect the ſeveral Parts after being divided; And

Alſo full DIRECTIONS how to feed, clean, manage and preſerve them at all Seaſons of the Year.

Likewiſe a COURSE of real EXPERIMENTS, performed by cutting theſe Creatures in every Way that can be eaſily contrived: ſhewing the daily Progreſs of each Part towards becoming a perfect POLYPE.

The Whole explained every where by great Numbers of proper Figures, and intermixt throughout with Variety of OBSERVATIONS and EXPERIMENTS.

By *HENRY BAKER*, Fellow of the *Royal Society*, and Member of the Society of *Antiquaries*, in *London*.

Rerum Natura nuſquam magis quàm in Minimis tota eſt.
PLIN. Nat. Hiſt. Lib. xi. c. 2.

LONDON:
Printed for R. DODSLEY, at *Tully's Head* in *Pall-Mall*, and
ſold by M. COOPER in *Pater-noſter-Row*, and J. CUFF,
Optician, in *Fleetſtreet*. 1743.

(Price bound Four Shillings.)

FIG. 8.

well-known "Buffon-Needham Theory") as well as his study of *Hydra* which he published in a volume of 222 pages under the title of "An Attempt towards a Natural History of the Polype: In a Letter to Martin Folkes, Esq; President of the Royal Society," London, 1743 (*cf*. Fig. 3).

> That curious Observer of Nature, Mr. LEEUWENHOEK, first took notice of this Animal, and the uncommon Way its young ones are produced, in the Year 1703 . . . but its more amazing Properties were reserved for the Inquisitive and happy Genius of Mr. TREMBLEY to discover, in the Year 1739 . . . observing it in some Respects to bear the Resemblance of a Plant, and in others of an Animal, he resolved, by cutting it in pieces, to satisfy himself, whether of the two it really was; and found, by this Trial, that, after a few Days, each Piece became a perfect Body, of the same Form exactly as *That* of which it had been only a Part: which Appearance would have determin'd him to conclude it to be a Vegetable, had he not discovered in it at the same time, a frequent Change of Figure, a Motion from place to place, a greedy and voracious Appetite, and a singular Dexterity in catching, mastering and devouring Insects and Worms. . . .
>
> In consequence of these Discoveries, he, ever since, has been making a Variety of such Experiments as none but his own fertile Invention would, probably, have contrived. These Experiments were performed in Sight of many of the Curious. . . . Some of these Creatures were likewise sent both to Mons. REAUMUR and YOU, lest any Difficulty of finding them, might prevent, discourage, or delay making the same Trials in *France*, or *England*, as himself had done at the *Hague*.[5]
>
> When Accounts of the extraordinary Properties of this Creature were communicated to you . . . it was never expected we should rest contented with their Accounts without making Experiments ourselves: *Nullius in Verba* being the wise Motto and establish'd Maxim of the ROYAL SOCIETY. But in respect to the Reputation of the ROYAL SOCIETY as well as to the Gentlemen who communicated these Discoveries, it became incumbent on us, as soon as they had sent the Insects over hither, to put them to a severe but speedy Trial, and from the Issue of our own Experience, either convince the World that these Gentlemen had been mistaken, or give our Testimony that what they affirm is true. This, SIR, was your opinion. . . .[6]
>
> YOU was, likewise, so obliging to favour me with three of your *Polypes*, very soon after their Arrival, with Intent that I should put them to the severest Test; and, to encourage and assist me in so doing, have frequently honoured me with your Company, and been yourself a Witness of my Proceedings.
>
> With these three *Polypes* I began my Experiments. . . . And I have gone on till this very Day repeating most of them several Times over, without finding any considerable Difference, but that of a much quicker Growth and Separation of the Parts cut to Pieces as the Weather became warmer. . . . Though it may not be improper to remark, that what by Divisions, Subdivisions, and the Creature's natural Increase, several hundreds have been produced by my first three, between *March* the twenty fifth, and the present fourth Day of *August*.

[5] "Natural History of the Polype," pp. 4–5.

[6] *Ibid.*, p. 201.

> These, however, were not all the Polype I have had under my daily Care and Inspection: for . . . Mr. ELLICOTT, F.R.S. gave me six *English* Ones, and . . . seven or eight green Ones, . . . which have also increased considerably. And, in *July* last, you favoured me farther with some of the longarm'd Sort, just then arirved from Mons. TREMBLEY.
>
> You, SIR, who know my Way of thinking, will not I am persuaded so far mistake me, as to imagine I am attempting, by this Essay, to vie either with yourself, or Mr. TREMBLEY; but it may not be improper to assure that Gentleman and the World, who are not so well acquainted with me, that I am as far from, as unequal to, such a Design; and that my real and only Motive to the many Experiments I have made, to the Care I have taken in propagating these Creatures, to the Readiness wherewith I have sent Numbers of them to *Oxford* and *Cambridge*, and dispersed them, as much as I have been able, amongst the Curious, and to this present Undertaking, has been to vindicate the Truth: which suffers sometimes for want of proper Means to prove it: and to display before Mankind, a new Instance of the amazing Power of the Creator.[7]

Thus the first book devoted to regeneration in animals appeared in England in 1743 and in a French translation in Paris in 1744, the same year that Trembley's famous "Mémoires pour servir à l'histoire d'un genre de Polypes d'eau douce" was issued at Leyden.

In view of the fact that Baker's work is not discussed in any, and the title of his book appears in the bibliography of only one of the recent volumes on regeneration, as a matter of record it seems worth while to give his list of experiments:

> Experiment I. Cutting off a Polype's Head; II. Cutting a Polype in two Pieces, transversly; III. A Polype cut in three Pieces transversly; IV. Cutting the Head of a Polype in four Pieces; V. Cutting a Polype in two Parts, lengthways; VI. Cutting a young Polype in two Pieces whilst still hanging to its Parent; VII. Cutting a Polype lengthways through the Body, without dividing the Head; VIII. A Repetition of the foregoing Experiment, with different Success; IX. Cutting a Polype in two Places through the Head and Body, without dividing the Tail; X. Cutting off half a Polype's Tail; XI. Cutting a Polype transversely, not quite through; XII. Cutting a Polype obliquely not quite through; XIII. Slitting a Polype open, and cutting off the End of its Tail; XIV. Cutting a Polype with four young Ones hanging to it; XV. Quartering a Polype; XVI. Cutting a Polype in three Pieces the long way; XVII. An Attempt to turn a Polype, and the Event; XVIII. Turning a Polype inside out; XIX. An Attempt to make the divided Parts of different Polypes unite; XX. A speedy Reproduction of a new Head; XXI. A young Polype becoming its Parent's Head; XXII. A cut Polype producing a young One, but not repairing itself.

In regard to Experiment XVIII., a repetition of Trembley's famous one in which he believed that he had permanently turned a Polype inside out, Baker says:

> Though I made several Trials before and since, I could never succeed

---

[7] *Ibid.*, pp. 6–10.

in turning *Polypes*, so well as in the above Experiment: which I impute to my Want of the Means Mr. TREMBLEY uses, as well as the Dexterity whereof he is Master: whose Account of his having turned many, and their living, thriving, and producing young Ones in that inverted State, I don't in the least doubt the Truth of. And when that Gentleman pleases to publish his own Method, which I should think myself unworthy of knowing if I endeavoured to take any of the Honour of it from him, most reasonable People, I believe, will be convinced.

The following extracts are from Baker's concluding remarks:

Having now, SIR, laid before you the most remarkable of my Experiments, in relation to the cutting *Polypes* asunder, and the Re-production of new Parts to make each Piece a perfect *Polype*; I shall entreat your Patience a little longer, whilst I add a few occasional Reflections. . . .

Though real Facts are incontestable Arguments, and no Reasoning seems necessary after so many repeated Experiments, there are certain Prepossessions, Prejudices and Humours among Mankind (arising from early imbibed Theories or Systems, according to which they have accustomed themselves to judge of Things) that make People sometimes disbelieve even what they see, are stronger than Reason, and will hardly be conquer'd even by the plainest Facts.

Hence it is that some have objected to the Reality of the *Polype's* being a living Creature, notwithstanding its moving from Place to Place, seizing its Prey, eating, digesting, and other Animal Functions: because its other Properties happen to be unsuitable to their Hypothesis of Life in general.

If the Animal Soul or Life, say they, be one indivisible Essence, all in all, and all in every Part, how comes it, in this Creature, to endure being divided forty or fifty Times, and still continue to exist and flourish?

Again: If animal Identity, say they, consists in Consciousness; and if every living Creature is sensible of Pleasure and Pain, or in other Words has a Consciousness, which most think a reasonable Supposition; when the *Polype* is divided into several Parts, all soon becoming perfect *Polypes*, where shall we find the Identity of the original *Polype*?

These Queries, I must acknowledge, I am wholly uncapable of resolving: but let those who tye themselves down to such Theories seriously consider, whether they believe themselves so perfectly acquainted with every living Creature God has made, and with all the Modes and Circumstances of the Life of each, as to be certain their Theories comprehend them all. 'Tis, methinks, a little presuming to restrain the Operations of Nature, or imagine that God has done nothing but according to certain Rules well known to us.

It is one great Part of Wisdom to know what we have Abilities for, and what Things are beyond our Power; that we may apply to the former, and avoid perplexing ourselves about the latter. How much valueable Time has been thrown away in framing whimsical unsatisfactory Schemes to account for the Operations of Nature, which might have furnished a great deal of profitable Knowledge, if spent in real Experiments on those self-same natural Operations?

When a Twig is cut off, and by planting in the Earth becomes a Tree of the Kind whereof it was a Part, can we account for its becoming so, any thing better than we can for the like Effect in a *Polype*? . . . The

> whole Difference is, we have known the One a long while, and the other is a late Discovery, which has not yet been noticed in our System of Animal Life. . . .
>
> 'Tis no great Wonder that Discoveries contrary to old and established Opinions should not at first be credited; but then, neither should they be absolutely rejected till Experiment has been made whether they are true or false.
>
> Those that know the most, are most sensible how little they know in comparison of what is yet unknown, and therefore consider Things with Modesty and Candour: but Ignorance cries out at once, it cannot be:—inconsiderately measuring the Powers of Nature by the scanty Compass of its own Experience, and more ready to reject the Truth than take the Pains to find it out.—A truly wise Man is so fully sensible how little he knows, and what Things he once was ignorant of, which he is now acquainted with, that he is far enough from supposing his own Judgment a Standard of the Reality of Things.

Trembley's and Baker's demonstrations of the potentialities of the polype, reflected by Linnæus naming it *Hydra,* and Hill, *Biota,* on account of the "strong principle of life with which every part is endued," created considerable excitement in scientific, philosophical and literary circles. They apparently had forgotten that Aristotle observed that there are animals as well as plants which propagate themselves by shoots; and on cutting one of these animals, the pieces which before comprised altogether but one animal become suddenly so many distinct individuals. And further that the soul in animals is in effect but one, though "multiplied in its powers as in plants." Or that St. Augustine relates in his "De Quantitate Animæ" that a friend in his presence cut a 'polype' in two, and immediately the two parts betook themselves to flight in opposite directions![8]

Henry Baker's original observations undoubtedly do not entitle him to a place among the leading pioneers with the microscope—for most of his observations were made apparently at random without any particular purpose in view and none of his discoveries is of great significance—while some of them are not discoveries at all, as Hill,[9] in his animadversions on the Royal Society, takes especial pleasure in pointing out. But Baker's books, appearing at a time when the effects of the first glimpses into the "world of the infinitely little" were slowly permeating into general culture, had a considerable influence on the popularization of microscopic work and the general recognition of its practical value, as well as in stimulating microscopic research.

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[8] Adams, "Essays on the Microscope," 2d edition, London, 1798.

[9] *Review of the Royal Society,* London, 1751.

"Baker on the Microscope" was something new—a product of the time—though, in a way, a descendant, with modifications, of Joblot's work on animalcules and microscopes[10] first published during the Leeuwenhoek period, and later reprinted with but slight change in 1754, when Baker's works were among the "best sellers." "Baker" was the humble forerunner within the limits of less than 800 octavo pages of our present-day manuals on bacteriology, protozoology and microbiology in general as well as of manuals on the microscope. Its success soon brought many competitors into the field, both in England and on the Continent—competitors such as the works by Adams which increased in bulk with each edition—but none of them approached it in originality of treatment, quaintness of expression, philosophical insight or the contagious enthusiasm of its author for things that are small.

"Baker on the Polype," the first volume ever devoted to regeneration in animals, gradually sank into oblivion on account of Trembley's classic memoir, but not without having exerted a considerable influence in England in stimulating experiments and speculations on life phenomena as "exhibited by that wonderful fresh-water insect the Polype."

> It has been said of Mr. Baker, that he was a Philosopher in little things. If it was intended by this language to lessen his reputation, there is no propriety in the stricture. . . . He was an intelligent, upright, benevolent man much respected by those who knew him best. His friends were the friends of Science and Virtue: and it will always be remembered by his contemporaries, that no one was more ready than himself to assist those with whom he was conversant, in their various researches and endeavors for the advancement of knowledge, and the benefit of Society.[11]

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[10] "Descriptions et usages de plusieurs nouveaux microscopes, tant simples que composez; avec de nouvelles observations faites sur une multitude innombrable d'insectes, & d'autres animaux de diverses especes, qui naissent dans des liqueurs préparées, & dans celles que ne le sont point." Paris, 1718.

[11] Biographia Britanica, 2d edition, London, 1778.

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OBSERVATIONS ON THE RELATION BETWEEN SUCKLING AND THE RATE OF EMBRYONIC DEVELOPMENT IN MICE

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INTRODUCTION

The present paper embodies the results of two separate but simultaneously conducted experiments, both designed to elucidate further the problems presented by the lengthened gestation period in mice which are pregnant and at the same time suckling a previous litter. Daniel ('10), working with such animals, obtained results which showed an almost constant relation of one day added to the gestation period for each animal suckled; the present writer ('16), however, in a somewhat similar series of experiments, obtained results which showed no correlation between the length of the gestation period and either the number of young suckled or the number of embryos carried (cf. also table 3). This work did, however, reveal the fact that when more than two young are being suckled the implantation of embryos instead of occurring on the fifth day after fertilization usually is delayed until the fourteenth day, during which period the blastulae lie free in the lumen of the uterus. The cause of this delayed implantation was tentatively stated in that paper to be due to an inhibition of some sort exerted by the fully activated mammary glands upon the uterine mucosa. The testing out of this hypothesis was the purpose of the following experiment.

INTERRUPTED SUCKLING AND THE RATE OF EMBRYONIC DEVELOPMENT

The program for this experiment was to take healthy female mice which had just given birth to litters, pair them for twenty-four hours with healthy males, and then remove all but one of the suckling young at intervals varying, with different females, from one to thirteen days. One young animal was left in each case to avoid too violent a reaction upon the lactating organs, and the presence of a solitary suckling animal has been found to have no influence upon the length of the gestation period. The females were all killed on the thirteenth day after the birth of the suckling young, and any embryos then present were sectioned and their age determined by reference to a check series of known age from non-suckling females (Kirkham, '16). The results of this experiment are set forth in table 1 and should be studied in two groups. The first group, cases when the full litter was suckled one to six days, embraces a period before the next set of embryos were ready to implant, while the second group, the remainder of the table, covers an interval when the blastulae were being in-

TABLE 1

Data of all mice used in an experiment to determine the effect on developing embryos of removing all but one of the suckling young. Unless otherwise stated. the full number of young born were suckled until removed for the purpose of the experiment. Stage of development of embryos is in terms of actual age of similar embryos from non-suckling females where thirteenth day post-partum equals twelfth day of embryonic development. All the females were killed on the thirteenth day after the birth of the suckled young

SERIAL NUMBER	DAYS SUCKLED	NUMBER OF YOUNG	NUMBER OF EMBRYOS	STAGE EMBRYONIC DEVELOPMENT	REMARKS
				days	
J 22	1	6	6	7	
J 21	2	7	6	11	
J 26	3	6	1	12	1 young died
J 27	3	6	8	7	
J 24	4	7	3	8	
J 25	4	6	6	10	
J 18	5	10	7	11	
J 17	6	7	11	8	
J 28	6	5	5	10	
J 29	6	4	6	10	
J 16	7	8	12	6	1 young died
J 12	8	12	10	7	
J 13	9	5	6	6	
J 10	10	7	9	6	
J 11	11	7	4+	4	1 horn uterus lost
T 14	13	5	9	4	

hibited from implanting. The importance of this grouping is apparent when one considers that in the first group of cases in every instance an interval of from a few hours to several days intervened between the end of suckling by the full litter and any readiness of the embryos to implant. Embryos in the second group, on the contrary, were presumably all prepared, before the removal of the suckling young, to implant themselves, and did so as soon as the inhibition acting upon the uterine mucosa fell below a certain minimum.

Analysis of the data, with these facts in mind, reveals the interesting fact that while some sets of embryos (J 18, 21, and 26) are as fully developed as control series when no young were being simultaneously suckled, others show a lag in development of from one to four days. No such diversity of results has been found by the writer in series of embryos from non-suckling females, and furthermore, it is evidently not directly correlated with the number of young suckled (cf. J 26 and J 27), the number of embryos (cf. J 21 and J 22), or with the combination of these two numbers (cf. J 18 and J 27). There remains the explanation that the irregularity is due to an individual variation either in the strength of an inhibitory influence exerted by the mammary glands upon the uterus or in the susceptibility of the uterus to such influence. Evidently some individuals are so constituted metabolically that the inhibition is rapidly and entirely neutralized, so that if even a short interval elapses between cessation of full mammary activity and the arrival of the eggs in the uterus, implantation and further embryonic development proceed as though no young had been suckled, while in other individuals an even longer interval still leads to delay in embryonic growth. The possible existence of such an inhibitory influence of the activated mammary glands upon the uterine mucosa has previously been shown by the experiments of Adler ('12), who found that repeated injection of extracts of mammary gland into pregnant guinea-pigs and rabbits arrested the development of embryos and often produced abortion.

CONSTANT NUMBER SUCKLING AND THE DEVELOPMENT OF EMBRYOS

The second method of attacking the problem of prolonged gestation in suckling female mice was to determine whether or not, if the number of young suckled was constant, the stage of embryonic development could at any given time after fertilization be theoretically determined. Four was chosen as the constant number, since it was neither the smallest size of litter known to prolong gestation nor, on the other hand, was it such a large number as to prevent the use of nearly all of the available material.

Females who had just given birth to litters of four or more young had the male already present removed, together with any excess number of young. This was done the morning following the birth of the litter, and at varying intervals thereafter the females were killed, the eggs or embryos obtained from these females were sectioned, and their stage of development determined, the assembled data being shown in table 2.

The first notable feature in table 2 is the delay in implantation, no implanted embryos being found before the twelfth day after ovulation, a confirmation of work published by the writer in a previous paper ('16). After the twelfth day of gestation there appears the same lack of correlation, as noted above in connection with table 1, between their stage of development and the time of ovulation, and since if degeneration is going to occur it always takes place at the stage of four or five days' development, this possible factor can be ruled out. In other words, we have here proof of the fact that the irregularities in the rate of development of embryos in females which are simultaneously pregnant and suckling is due, only in small measure, if at all, to the number of young suckled, for if the number suckled and the rate of embryonic development were correlated the data in table 2 should indicate a regular correlation between stage of development and age of embryos, and such is not the case.

The explanation of this failure to correlate the ratio of embryonic development in suckling females with either the size of the

TABLE 2

Data of all mice used in an experiment to determine the effect on developing embryos of the suckling of a fixed number of young. In this experiment all litters within twelve hours of their birth were reduced to four young. Stage of embryonic development in terms of actual age of similar embryos from non-suckling females

SERIAL NUMBER	SIZE OF LITTER	AGE OF SUCKLED YOUNG	STAGE EMBRYONIC DEVELOPMENT	DELAY
		days	*days*	*days*
T 75	4	3	2	0
T 11	4	4	3	0
T 70	4	5	4	0
T 24	4	6	4	1
T 63	8	9	4	4
T 67	8	10	4	5
T 43	4	11	4	6
T 42	4	12	4	7
T 66	5	13	4	8
T 62	8	14	4	9
T 15	4	15	10	4
T 54	5	16	7	8
T 65	6	17	9	7
T 60	5	18	5	12
T 56	4	19	6	12
T 68	8	20	14	5
T 73	4	21	18	2
T 61	5	22	9	12
T 57	6	23	13	9
T 59	8	24	11	12
T 71	7	26	17	8
T 72	9	26	19	6

litter or with any other factor is to be sought in the first part of this paper, where the irregular influence of the termination of suckling by a full litter is conclusively shown. Add to this the fact that under normal circumstances the suckling young begin to eat solid food at about the time implantation occurs in females which are simultaneously pregnant, and the evidence here presented all indicates that after full suckling ceases, whether by removal of the litter or weaning matters not, the inhibition to implantation is withdrawn or overcome at a widely different rate in different females. Therefore, even when the number suckled is the same, the sets of embryos show no constant rate of develop-

TABLE 3

Data regarding the length of the gestation period in mice simultaneously pregnant and suckling young. The gestation period in non-suckling females averages twenty days

NUMBER SUCKLED	GESTATION PERIOD OF EMBRYOS	DELAY	REMARKS
	days	*days*	
1	20	0	A male was present with each female when the young were born, and in each case the male was removed 24 hours later
1	20	0	
2	19	0	
2	20	0	
3	20	0	
3	29	9	
3	30	10	
4	31	11	
4	30	10	

ment, nor, as a consequence, is the period of gestation a matter that can be figured out in advance, except with broad limitations (table 3).

SUMMARY

1. In mice simultaneously suckling and pregnant the removal of all but one of the suckling young at any time during the first six days after the birth of the suckling litter leads in some instances to implantation of the embryos as soon as they reach the uterus; in other instances the implantation is more or less delayed. These varying results can be correlated neither with the time of removal nor with the number of young taken away.
2. In mice simultaneously suckling and pregnant the removal of all but one of the suckling young at any time from seven to fourteen days after the birth of the suckling litter regularly results in implantation being delayed, but the exact extent of this delay can be correlated neither with the exact time of removal nor with the number of young removed, although removal during the earlier part of the period in question does hasten implantation as compared with the time required if the young had continued to suckle.
3. The facts in the above paragraphs justify the statement that full activity of the mammary glands is the chief cause of

delayed implantation in the case of mice which are suckling young, and also that this influence of the mammary glands is subject to marked individual variation.

4. The suckling by pregnant female mice of the same number (four in this experiment) of young will not necessarily lead to either synchronous development of embryos or the same length of gestation periods. The only explanation that can be offered at the present time for this lack of uniformity is the individual variation, noted in the preceding paragraph, of the inhibition from the mammary glands or in the strength of the counteracting forces, probably due to metabolic idiosyncrasies.

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DEMONSTRATION OF EPITHELIAL MOVEMENT BY THE USE OF VITAL STAINING, WITH OBSERVATIONS ON PHAGOCYTOSIS IN THE CORNEAL EPITHELIUM

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FOUR FIGURES

Though the cornea of the adult frog is thin and transparent enough for the observance of epithelial movement, it is not an easy matter to note in detail every part of the process. For this reason certain vital stains were used in the present experiments.

In the amphibians, vital staining can be produced either by introducing the dyes into water in which the animals swim, or by injection, though the latter procedure is not practicable in the case of corneal epithelium, owing to its lack of blood-vessels The stained tissue may then be explanted. Another method is to add the dyestuffs to the culture medium containing the unstained tissue.

Several papers have appeared dealing with the different phases of this subject (Lewis and Lewis, '15; Russel, '14).

For the purpose of the present work it is necessary that the dyes should not have any deleterious effect upon the epithelial cells in order that they may remain capable of maintaining their normal activity. The staining must be produced easily and last for a considerable time.

That the epithelium of amphibians (especially larvae) is tingeable by various dyestuffs without affecting the organism or tissue elements to any marked degree has been shown by various observers (Fischel, '01). Whether vital staining may be employed in order to demonstrate moving epithelium in the culture is, however, another question.

A. METHODS OF VITAL STAINING

1. Methods of staining by immersing the whole animal in water containing dyes

a. Neutral red. Neutral red, which was first used by Ehrlich for vital staining, was considered as probably being the most suitable for the present purpose, and this proved to be the case.

In the majority of the experiments "neutral red for vital staining (Ehrlich)" was used, and the frogs were subjected to various concentrations of the dye for various lengths of time.

The experiments may be divided into two groups. In the first the animals were subjected to a relatively concentrated (1: 20,000) neutral red solution for a short time (one to five hours), and in the second they were kept in a more dilute solution (1: 100,000 to 1,000,000) for a much longer period (one-half to four days). The frog was then carefully washed in running water, the tissue removed and prepared in a manner similar to that used in previous experiments (Matsumoto, '18). Both modes of staining are adapted to the study of the movement of epithelium.

Though the frog could be stained an intense red without doing it any apparent harm, still the dye proved to be more or less harmful to the cells. In fact, an earlier degeneration of the explanted tissue due to the injury of cells in such preparations was frequently seen. As it was desirable to stain the tissue in such a manner that the granules would be just evident enough for observation, weak solutions (such as 1: 100,000 to 1: 200,000; ten to twenty-four hours) were preferable. The use of the more rapid staining, at times, brought on the disadvantage of having the granules appear unevenly distributed.

The arrangement of the neutral red granules shows a more or less marked difference in the several cell layers of the corneal epithelium. Observing from the upper (outer) surface of the corneal tissue in the culture, we see the following:

1. The outermost layer (fig. 1, *d*) consists of flat polygonal cells with large nucleus and unstained fine refractive granules in the cytoplasm. These cells, which may be present all over the

original epithelial surface or may be absent here and there, do not usually exhibit neutral red granules and show no activity.

2. The next layer (fig. 1, *c*), which is the uppermost layer in some preparations, consists of cells which contain in the cytoplasm the most minute red granules, almost uniform in size. These appear in abundance after prolonged intense staining. The contour of the individual cells is hardly visible at first. In some instances, especially when the rapid method of staining was employed, larger granules appeared in the cells so abundantly that they almost filled the cell body, leaving only a clear round or oval space in the center, occupied by the nucleus.

3. The basal cells (fig. 1, *a*), which are evidently smaller than those of the upper layers, exhibit, as a rule, distinct and much larger granules, not very uniform in size, and often more numerous on one side of the nucleus than on the other.

4. Between the two latter layers frequently another layer of cells (fig. 1, *b*) is visible. These are intermediate in size between the cells of the basal layer and those of the layer above, and as regards the arrangement of the colored granules show a resemblance to the cells of the second layer.

Furthermore, between the second and third layers there were found a number of deeply red stained bodies, round or irregular in shape, some of which showed a more deeply stained red spot (one of these is shown in fig. 1, *a*, and another in fig. 2, *a*). The origin of these bodies is unknown.

When the culture is observed several hours or more after the preparation, the cells of the upper and basal layers are easily distinguishable according to the distribution of granules in their cytoplasm, and this becomes more marked as the tissue becomes more translucent. The individual granules were sometimes observed to coalesce gradually, changing in shape.

When the tissue is intensely stained, the cells of the connective tissue and of the posterior endothelium also show distinct granules which are usually not so abundant and are more or less distinguishable from those of the epithelium. The nucleus remains unstained throughout.

Cultivated in vitro, the corneal epithelium, when stained with neutral red, showed a condition entirely similar to that seen in unstained preparations. The cells exhibited practically the same degree of activity, though at times they degenerated a little earlier than in the control preparation.

By this method, the cell movement along the tissue, which took place, as a rule in the fluid or semifluid culture medium, could be easily detected. That the spreading epithelium was, as a rule, two and sometimes three layers thick, was clearly demonstrated (fig. 2, *a*), while in an unstained preparation it was rather hard to determine whether they were one or two cells in thickness.

As the epithelium spread out and the individual cells became very flat and thin, a change in the arrangement of the granules took place. Then the granules of the basal cells, which were rather irregularly distributed at first, frequently assumed a crescent-shaped arrangement (fig. 2, *a*). If the staining was light, the tiniest red granules in the cells of the surface layer became faint relatively earlier. As a rule, the hyalin processes of the cells on the border did not exhibit any red granules. In general, after all activity of cells ceased, the red granules faded out and fatty granules increased, whereas the nucleus remained unstained. The same was true of the isolated epithelial cell.

So far as our observations go, there was in general a direct relation between the cell activity and presence of neutral red granules, although of course the intensity or richness of the granules was not always parallel with the cell activity.

b. Some other dyes. In the corneal epithelium subjected to 'Nile blue sulphate,' the granules were beautifully stained. This dye was found, however, to be injurious to the cells, and even the solution 1:200,000 in most instances caused more or less injury to the epithelium, though not enough to interfere immediately with the cell activity, since in many cases the cells continued to move. Disintegration of cells took place earlier, and a preparation which showed intense granulation never lived so long as the control culture (unstained or stained with neutral red). In addition to this, in the preparation subjected to the dye, fine violet crystals appeared in the cells or in the medium. Nile blue, therefore, does not seem to be a very favorable dye for the corneal epithelium.

Neither is 'methylene blue (rectif.)' suitable for the purpose, as it fades too easily.

So far as our observations go, 'gentian violet' did not give a satisfactory result. Even the weakest solution, which stained the cells, caused their death.

'Brilliant cresyl blue 2 b' and 'methyl green' did not stain the granules clearly, nor did 'indigo carmin,' 'toluidine blue,' or 'litmus' give positive results.

2. Staining of the cornea by administration of dyes in the conjunctival sac

Arnold ('00) demonstrated neutral red and methylene blue granules in the frog cornea by conjunctival administration of the dyes in the form of fine powder. In this way typical granules appeared in the cells, often causing injury. No experiments of this kind are recorded in the present paper.

3. Staining of excised cornea

In some experiments the cornea was first excised from the animal and then stained. The tissue, after removal, was immersed in physiological saline solution containing neutral red, freshly prepared, so that the epithelium became intensely red, and could later be cultivated after thorough washing. The technique is simple and is useful in some particular instances. Usually, however, crystals appeared in the culture, when this method was employed.

4. Staining by addition of dyes to the culture medium

Both neutral red and Nile blue, freshly dissolved in salt solution, were experimented with. They are easily soluble in pure water, but not so easily if the water contains any salt (NaCl). The appearance of crystals and the uneveness of the staining proved this method to be an unsatisfactory one. Staining of nucleus and granules by the use of gentian violet failed. Methylene blue stained granules, but it could not be employed for the purpose on hand.

B. PHAGOCYTIC PHENOMENON OF THE CORNEAL EPITHELIUM

Though the experiments on this line have not yet been completed, some interesting phenomena will be described here.

It was noted in the course of this work, that not infrequently in the preparations of cornea, where the pigment of the iris or chorioidea was accidentally mixed with the epithelium, the cells of the latter, originally not pigmented, took up the pigment, becoming gorged with it. This was easily demonstrable, but more markedly when the pigment of the eye was finely teased and put into the culture medium with the corneal epithelium. The cells of the moving border, especially, took up the dark pigment abundantly.

That the melanin granules are really taken up in the cytoplasm of cells, was clearly demonstrated in the preparations which were first vitally stained with neutral red (fig. 3). That those pigmented cells were not the originally pigmented epithelium of eye, accidentally mixed in the preparation, is beyond doubt. The epithelial cells were able to take also melanin which was previously heated or boiled.

The arrangement of the pigment granules in the cytoplasm was characteristic, resembling that of the neutral red granulations. The cells of the basal layer were often abundantly packed with the pigment, and those of upper layers were able to ingest it, too.

If in the epithelial cells which are originally not pigmented, abundant melanin is taken up, it is extremely difficult to distinguish them from the original pigment cells.

The possibility of epithelial phagocytosis has been considered by Riehl ('84); somewhat later, Rabl ('96) also took up the question and found that the epithelium of adult salamanders (S. maculosa) is able to take up carmin injected subcutaneously. In cultures of carcinoma and sarcoma, Lambert and Hanes ('11) demonstrated that carmin granules are taken up by the cells. Carrel and Burrows ('11) observed a similar phenomenon in cultures of sarcoma.

The melanin problem represents one of the most interesting questions in dermatohistology and experimental dermatology and is a much discussed subject. I should not go, of course, so far as to say that the so-called 'Einschleppungstheorie' of melanin is generally accepted, though the fact stated above shows the possibility of its correctness.

Though the observations here recorded have been made only upon cells in culture, still it may not be impossible that the same occurrence takes place in vivo under certain conditions. At any rate, the phagocytic action toward melanin of epithelium of the adult frog in culture is here definitely shown.

Therefore, it is not quite safe, in the culture of pigmented epithelium, such as iris, to consider the pigment granules as the peculiar possession of that epithelium, because the original non-pigmented epithelium can ingest pigment granules. The study of a complex and peculiar cell, such as the pigment cell, requires great care in the methods used and in conclusions drawn.

Carmin, which was ground into fine powder, was also taken up into the cell bodies, which then showed beautiful carmin granulation. For this purpose the use of tissue, vitally stained with Nile blue, offered great advantage.

The arrangement and distribution of the granules was entirely similar to that of the melanin; the particles were arranged with considerable uniformity around the periphery of the cytoplasm.

As figure 4 shows, corneal epithelium, cultivated in plasma (or serum), containing both melanin and carmin, exhibits beautiful melanin and carmin granulations which can be preserved safely. Examination of such preparations in serial sections demonstrated the intracellular and perinuclear position of the granules. That they are in the cell bodies may be placed beyond doubt even by the careful observance of fresh preparation. Furthermore, such preparations have the advantage of enabling the observer to watch how cells with ingested granules may become detached from the main mass and move off in the culture medium.

Fine granules of Chinese ink or minute foreign bodies accidentally mixed in the culture were also taken up by the epithelium.

In regard to this most interesting phenomenon of epithelial phagocytosis, our present knowledge is very deficient. A strict differentiation of the epithelial movement from that of leucocyte may not be possible in the case of the tissues of the frog living in vitro. Not only in their movements, but also in the phagocytic phenomena (with respect to foreign bodies) do they show a certain degree of resemblance.

The question of the said phenomena in the warm-blooded animals, as well as their bearing upon bacteriology and serology, will be considered later.

I wish to thank Prof. R. G. Harrison for the direction and support which he has given to this work.

C. SUMMARY

The epithelium of the cornea of the frog which was vitally stained with neutral red and Nile blue exhibits characteristic granules in the cytoplasm.

If properly stained, the granules exist through the entire period of cell activity, without practically affecting the cells, and facilitate the observance of cell movements.

Phagocytic phenomena of the corneal epithelium with reference to melanin, carmin, etc., are definitely demonstrated.

The distribution and arrangement of melanin and carmin granules, if finely powdered, show a certain degree of resemblance to that of neutral red and Nile blue.

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PLATE 1

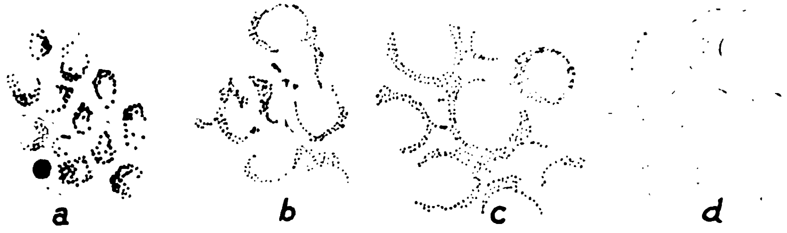
EXPLANATION OF FIGURES

1 Experiment 202. C. 11. Neutral red granules in different layers of the corneal epithelium (adult frog) cultivated in plasma, 12 hours old. *a*, Granules in the basal cells; *b*, cells of a layer often recognizable between *a* and *c*; *c*, granules in the cells of upper layer; *d*, cells of the uppermost layer, which show no activity. × 450.

2 Experiment 206. A. 3, showing neutral red granules in the moving epithelium, *a*, endothelium, *b*, and below, those of connective-tissue cells; *c*, 24 hours old. Culture in plasma. Movement (from left to right) of the ameboid border of the epithelium, *a*, on the endothelial surface *b*, is demonstrated. That the moving epithelial membrane consists at least of two layers of cells is readily confirmed by the types of granulation. Note the crescent-shaped arrangement of granules in the basal cells. On the right, *b*, those of the endothelium are represented; the contours of the cells are not visible. Connective-tissue cells (*c*), the contours of which are visible, show granules in their fine processes, too. As a rule, the hyaline processes of the epithelium do not show any granules (see also figs. 3 and 4). The tissue was intensely stained (four days in the dye 1: 200,000). × 250.

3 Experiment 226. A. 2, showing phagocytic phenomenon of the corneal epithelium, which are previously stained with neutral red; 24 hours old. Resemblance of arrangement of melanin granules to that of neutral red is noticeable. *a*, Drawn from a part of epithelium, moving on the endothelial surface of cornea; *b*, epithelial cells of actively moving border. × 450.

4 Experiment 236. 12. Phagocytosis of the epithelium to melanin and carmin; 18 hours old. Culture in autoplasma, in which fine powdered carmin and fresh melnin of iris were added. Drawn from a part of moving epithelial rim. × 450.



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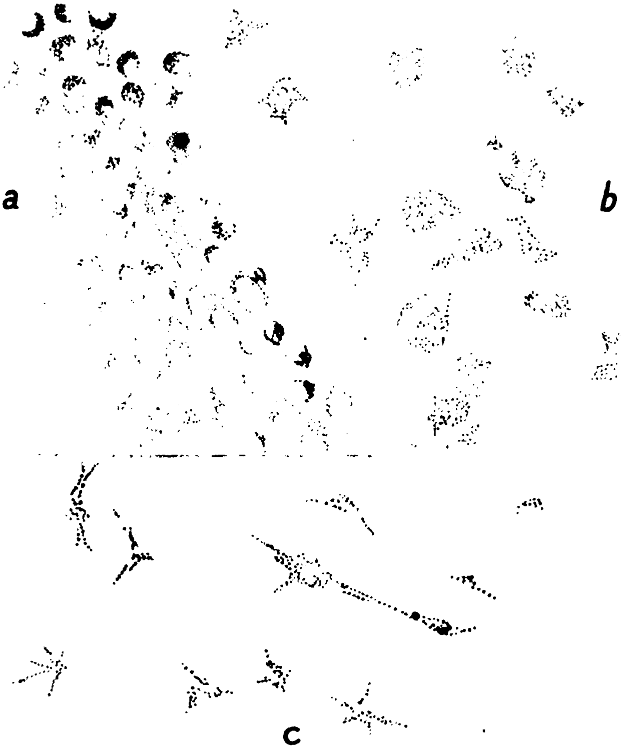

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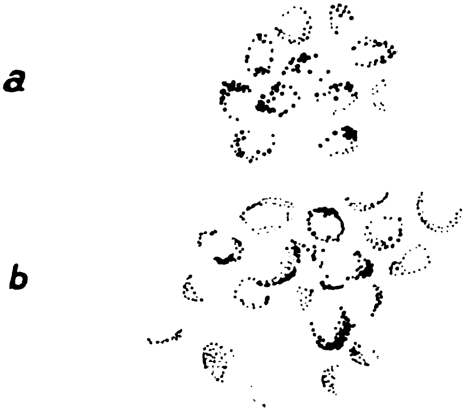

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THE REACTION OF SELACHII TO INJECTIONS OF VARIOUS NON-TOXIC SOLUTIONS AND SUSPENSIONS (INCLUDING VITAL DYES), AND TO EXCRETORY TOXINS

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TWENTY-SEVEN FIGURES (SIX PLATES)

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INTRODUCTION

The present study grew out of an attempt to determine the function of the digitiform gland, the development of which has recently been described (Hoskins, '17). This gland is peculiar to selachians, being found in no other group of animals, although in Chimaera, there is present in the wall of the posterior portion of the intestine a group of cells which may possibly correspond to it (Disselhorst, '04).

Nearly every reasonable function has been ascribed to the digitiform gland, but usually from morphological or hypothetical rather than physiological evidence (Hoskins, '17). The gland is a compound tubular structure with a large central lumen or duct which empties into the intestine about half way between the spiral valve and the cloaca. It is suspended in the mesentery above the intestine. Cytologically, the gland is not unlike the kidney tubules, and this, together with the fact that its secretion is discharged into the intestine posterior to the region where digestion occurs, leads to the expectation that it has an excretory function. The fact that the gland appears to be the same in both sexes argues against the probability that it is accessory to the sexual apparatus.

Another part of the work is a study of the function of the kidney (mesonephros) compared with that of mammals (metanephros). It is common knowledge that the former is a less efficient excretory organ than the latter, but the subject has never been completely studied experimentally. A study is made also of the excretory function of the liver, which is of considerable importance in selachians, on account of the inefficiency of the kidney. In selachians the liver undergoes fatty metamorphosis to such extent that nearly every cell appears filled with fat, yet the organ is able to excrete solutions and particles freely and in large amounts.

Denis ('13) has shown that dogfish are able to withstand large doses of excretory toxins, but this author did not study the problem histologically. The question of vital staining is considered here only incidentally and no attempt is made to describe completely the reaction of the dogfish to vital stains. These substances were used primarily as an aid to the study of the excretory function.

Most of the experiments were performed at the Marine Biological Laboratory at Woods' Hole, Massachusetts, in quarters provided us through the kindness of the Department of Zoology of Yale University and of Dr. Frank Lillie. The accompanying photomicrographs were made at the Osborn Zoological Laboratory of Yale University. Two experiments were done at the

Aquarium in New York. We desire here to express our thanks to the Zoology Department of Yale University, to Dr. Lillie, and to Dr. C. H. Townsend, of the Aquarium.

MATERIAL AND METHODS

The animal used in most of the experiments is the dogfish Mustelus canis, which is fairly abundant at Wood's Hole. In the two experiments performed at the New York Aquarium we used Acanthias. In all about seventy-five animals were made use of. These were of both sexes and of various sizes, but most of them were adult males.

The study was divided into three phases; *a*) the reaction to non-toxic solutions; *b*) the reaction to suspended particles, and, *c*) the reaction to excretory toxins. The solutions used are indigo-carmine, potassium iodide, dextrose, sucrose, phenosulphonepthalein, and Weed's potassium ferrocyanide-iron ammonium citrate solution. The supensions are carmine, Congo red, neutral red, and trypan blue. The toxins are potassium chromate, tartaric acid, and uranium nitrate. All were prepared with sea-water, which is isotonic with the blood of dogfish. In preparing Weed's solution, sea-water, diluted one-third, was used.

Injections were made either into the muscles of the tail and back or intravascularly. In the latter method the large vessels of the pectoral fins were used. A small wooden peg may be used for a hemostat and is very convenient as it can be easily removed for repeated injections. Intravascular injections may be made, also, directly into the sinuses of the head.

A simple device for holding the animals during the experiment may be made from a board 4 inches wide and 2 feet long. A long hole is made near one end for the dorsal fins and a row of nails driven partly in along each side of the board. The animal will be securely held on this if a cord is laced from the nails back and forth across its body. A tube of running water inserted into the animal's mouth will provide for respiration during an operation. The entire apparatus with the fish in normal position may be immersed in the aquarium if it is necessary that the animal be kept immobilized. In collecting urine from an immersed animal the receptacle can be sealed tight and weighted to keep it under the water. A long glass tube leading into it will permit the air to excape as the urine accumulates. If a long rubber tube is used to connect the cannula with the collecting bottle the animal can even be permitted to swim about in the aquarium while the urine is being collected. Denis ('12) also describes a good method for collecting urine.

The duct of the digitiform gland enters the intestine at a peculiar angle, being completely bent on itself. In order to insert a cannula into the duct it is best to reach into the intestine with a pair of blunt forceps and turn the gut inside out through the cloaca. This straightens the duct and a cannula can then be introduced directly into it. No anaesthetic is required in operations as the animal will lie quietly in the apparatus described above and seems insensible to pain. Antiseptics were also found to be unnecessary in operations.

EXPERIMENTS

1. Solutions

A. Indigo-carmine. 1. An adult male dogfish was injected intramuscularly with 5 cc. of 0.5 per cent indigo-carmine. This appeared in the urine in fifteen minutes. It did not appear in the stomach, spiral-valve or digitiform gland contents, at least in detectable quantities. The bile was bluer than normal.

2. The experiment was repeated with a female, and similar results obtained.

3. Two animals, a male and a female, each received 5 cc. of the above solution intravenously. It was recovered from the urine of each after eight minutes.

4. A female was injected as in '3' and permitted to live five hours, at which time the urine was still dark blue in color.

All five of the above-mentioned animals were autopsied at various times after injections, varying from a half-hour to five hours. All secretions examined gave the same reactions as in

'1.' Also in every case the anterior portion of the kidney and liver was of a light blue color. The posterior portion of the kidney was dark blue and a dissection of the liver showed the bile ducts to be colored blue rather than the usual green. The other organs of the body cavity were not distinctly blue in color.

Glands were fixed in absolute alcohol, but the dye soon disappeared either by fading or going into solution. Sections were made and examined microscopically, but no blue granules were seen even though the gross specimens were blue. The dye was probably impure and hence dissolved out in the alcohol, as was found to be true in some cases by Heidenhain ('83) working with other animals.

B. Potassium iodide. An adult male fish received 10 cc. of a 3 per cent solution of potassium iodide intravenously. After three and a half hours tests were made with nitric acid and starch (Hawk, '16), and the iodide was found to be present in the urine in the gastric and intestinal fluids, the bile, and the secretion of the digitiform gland.

C. Dextrose. An adult female fish received intravenously 15 cc. of 25 per cent dextrose solution. At the end of one hour the urine was negative to Benedict's sugar test (Hawk,'16), but was positive after four hours and negative again after nine hours. At the latter time the bile gave a positive reaction, but the secretion of the digitiform gland reacted negatively.

D. Sucrose. The animal received intravenously 15 cc. of 30 per cent sucrose, and after six and one-half hours was autopsied. The reaction to Benedict's test was positive in the case of the urine, bile, and blood serum, but negative with the gastric and spiral-valve contents. The digitiform gland secretion was not tested.

E. Phenosulphonepthalein (Rowntree and Geraghty, '12). 1. One and two-tenths cc. of this pthalein was injected intramuscularly. It appeared in the urine in fourteen minutes. At autopsy it was not present in detectable amounts in the stomach, intestines, or digitiform gland.

2. An animal received intravenously 1 cc. of pthalein. Autopsy was performed after one hour. At this time the pthalein

was present in the urine, bile, and in the stomach and spiral-valve contents. The secretion of the digitiform gland and an extract of the spleen were negative.

3. An adult fish received 1.4 cc. of pthalein. The urine was collected for five and a half hours and the bile in the gall-bladder was removed at the end of that time. The urine, bile, and 1.4 cc. of pthalein in graduated cylinders were each diluted with water, acidified and all compared (Rowntree and Geraghty, '12). By this comparison it was shown that approximately one-half of the injected pthalein had been recovered, and of this, about one-fourth was in the urine and three-fourths in the bile. The bile pigments interfere slightly with the test. A trace of pthalein was found in the serum, digitiform gland secretion, and in the stomach and spiral-valve contents. That in the latter two may have come from excreted bile.

4. An adult received 1 cc. of pthalein. A cannula was inserted to collect the urine and the animal was then permitted to swim about in the aquarium for four and a half hours in order that the excretion might not in any way be interfered with by the unnatural position of the animal in the holding apparatus that was used in the first three experiments. In this specimen as in the others more pthalein was recovered in the bile than in the urine. Pthalein was also found in the spiral valve, but not in the stomach.

F. Weed's solution (potassium ferrocyanide and iron ammonium citrate, Weed, '12). 1. A young male (0.8 kg.) received intra muscularly 7.5 cc. of Weed's solution and was autopsied after four hours. The urine and bile gave the Prussian-blue reaction with acid. The serum and the stomach and spiral-valve contents were negative to the test.

2. A young animal (0.5 kg.) received intramuscularly 9 cc. of Weed's solution, followed after five hours by 9 cc., and after twenty-two hours by 8 cc. Twenty-five hours after the first injection the Prussian-blue reaction was obtained with the urine, bile, and serum. The liver turned blue on standing.

3. A young female (0.5 kg.) received, as above, 10 cc. of the solution, followed by 5 cc. after four hours, and 5 cc. after seven

hours. Autopsy was performed eight hours after the first injection. The Prussian-blue reaction was obtained from the serum and urine, but not from the bile, or stomach and spiral-valve contents. Pieces of organs were fixed in 20 per cent formaldehyde acidified to 1 per cent with hydrochloric acid.

4. An adult (Acanthias) received four hourly injections of 6 cc. each of Weed's solution, and was autopsied four and a half hours after the first dose. Organs were fixed in absolute alcohol acidified as above.

5. An adult (Acanthias) received three injections of 9 cc. each of Weed's solution at hour intervals and was autopsied forty-five minutes after the last injection. Forty per cent formaldehyde acidified to 1 per cent was used as a fixative. Microscopic examinations were made of various organs from the animals, '3,' '4,' and '5' with the following results:

a. Digitiform gland. In all the specimens examined there is a central, distinctly blue zone, the width of about one-half the radius of the gland as seen in transverse sections. Microscopically, the blue granules are seen almost entirely within the blood-vessels, although a very few are found in the cells of the tubules, but none in the lumen. The granules are present in the capillaries and central venous sinuses in greater concentration than in the vessels of any other organ.

b. Kidney. The gross specimens are dark blue in color. Sections show many Prussian-blue granules in the blood-vessels and tubules. A very few only are present in the glomerular cavities. They are found in the cytoplasm of both the secretory and excretory tubules. Blue granules are found also in the connective tissue of the kidney.

c. Liver. The gross specimens are all light blue in color. In a few hepatic cells blue granules are seen and they are present in greater number in the ducts. This indicates either that the solution passed directly into the ducts, as was thought to be the case of trypan blue in bony fishes (Wislocki, '17), or else that the solution passed through the epithelium quickly and out of the ducts more slowly, thus becoming concentrated in the ducts. In the liver of animal '3' the endothelium and the leucocytes of the hepatic sinuses have a deposit of blue granules on or in them. This condition is not seen in other organs of the same animal nor in the liver of other animals.

d. Spleen. Blue granules are scattered throughout the spleen among, but not within the cells.

e. Spiral-valve, stomach, gills, and muscle. All these have blue granules in the blood-vessels and scattered through the connective tissue, but none within any cells. In gross free-hand sections the epithelium of the stomach and spiral-valve appears white, in striking contrast with the dark blue connective-tissue.

2. Suspensions

A. Carmine. Powdered carmine was used for coarse granules, although some of it seems to go into solution in sea-water. It will not all settle out on standing, cannot be separated out with ordinary filter-paper, and will even pass through a celloidin membrane. After passing through celloidin no granules can be seen with the ordinary microscope. This may not be in true solution, but if not it is practically so.

1. Filtered carmine after settling for several days was injected intravenously into an adult male dogfish. The animal received four daily doses of 12 cc. each and was autopsied eight hours after the last injection. Microscopic examination of the various organs failed to show the presence of any carmine granules, although the spleen appeared distinctly red after fixation. The granules were either too small and too much scattered to be seen or else the carmine was in solution and washed out in preparing the sections. The urine was not collected.

2. Ten adult animals were injected intravenously each with 10 cc. of a heavy suspension of powdered carmine in sea-water and autopsied after various periods as follows: 1½, 6½, 21, 24, 50, and 96 hours. This carmine was not filtered. Pieces of the various organs and tissues were fixed in formalin, sectioned in paraffin, and stained with haematoxylin, or examined unstained.

Urine was collected from several of the animals, but in only one specimen of it was any carmine found, and in that but one granule. This sample of urine also contained a few blood corpuscles and hence the carmine probably entered with the blood.

Microscopical examination. *a.* Digitiform gland. Carmine is present in the endothelium and leucocytes of the blood-vessels in this gland in all stages, but very little in the first (1½ hours), and less in all the sections than in the endothelium of the kidney, liver, spleen, and gills. In the 21-, 24-, and 50-hour specimens there is an occasional carmine granule seen in the cytoplasm of the tubules. These few granules may possibly have been dragged into the cells in the cutting of the sections and in any case are too few in number to be of any importance. None was seen in the lumen of the tubules.

b. Kidney. In the first two stages there is considerable amount of free carmine in the capillaries, much of it in large clumps filling the entire vessel, and a few leucocytes and endothelial cells contain carmine granules.

In later stages (21 to 96 hours) there is a gradual decrease in the amount of free carmine in the capillaries and there are no large masses of it present. There is a decreasing amount seen in the leucocytes and endothelium. A few sections have carmine granules in the connective-tissue cells. In only three tubules of the hundreds of sections examined are carmine granules to be seen in the cytoplasm. These few granules may have been carried in, in the preparation, and as in the digitiform gland are considered to be of no practical significance. Each of two tubules of one section contained a granule in the lumen, but one also finds blood corpuscles occasionally in the tubules and these carmine granules may have entered accidentally.

c. Liver. Carmine granules are seen in all stages after the first, in the leucocytes and endothelial cells of the sinusoids, in the hepatic cells, and in the normal pigment accumulations, which are very abundant in the liver of the dogfish.

Carmine was found in the bile in all stages after the first, as free granules, in cells resembling leucocytes and mixed with the normal pigment.

d. Spleen. Carmine is present in all the sections of spleen examined and in decreasing amounts in later stages (fig. 1).

In all stages carmine may be seen as granules free in the sinuses, and ingested by the endothelium of the sinuses and by splenic cells. The insert in figure 1 shows an endothelial cell that has extended a pseudopodial process and engulfed a very large granule. The figure also shows an endothelial cell filled with carmine and apparently about to be liberated as a free phagocyte (macrophage?). In many places the ingested carmine seems to be enclosed in vacuoles.

There is a decided increase in the number of mitotic figures seen in the spleen beginning with the 21-hour stage, indicating a leucocytosis.

e. Spiral-valve and stomach. In these organs there is an occasional phagocyte in the capillaries that contains carmine. There is no carmine outside the vessels.

f. Gills. The large sinuses which are interposed between the branchial arterial arches and the capillaries in the filaments are lined with an endothelium which is very phagocytic. This will be described in detail under 'Trypan blue.' In the earlier stages the gills were not examined, but sections of later stages (50 and 96 hours) show the endothelial cells of the sinuses to be engorged with carmine granules. Many of these cells have also been 'budded off' and lie in the channel as free phagocytes. This endothelium is doubtless one of the sources of circulating phagocytes in the dogsfish.

Even the arterial arches are lined with phagocytic endothelium especially along the side next to the sinuses.

g. Heart. The 1½-hour stage shows a few cells of the endocardium that are phagocytic to carmine. The heart in other stages was not examined.

h. Blood. In all stages the blood contains free carmine and granules ingested by phagocytes. The number of free granules decreases in the later stages.

B. Neutral red. Two series of experiments were conducted with neutral red and to some extent with different results. In 1916 the sample of neutral red used seemed to form a true suspension, as it could all be separated from the water with ordinary filter-paper.

1. An adult received intravenously 10 cc. of suspension of neutral red and was permitted to swim about in the aquarium. At that time the animal was immobilized, received an additional 18 cc. of the suspension, and its urine was collected for five hours without any of the neutral red appearing in it. Autopsy showed the bile to be reddish brown. This turned orange with alkali and dark lilac with acid. Serum gave a similar reaction, but the gastric fluid was negative. The suspension was prepared by stirring neutral red in sea-water and allowing the mixture to settle.

2. An adult was injected intravenously with 15 cc. of saturated neutral red suspension. The urine was collected for six hours and was at all times free of the dye. Autopsy showed neutral red in the bile in a considerable amount.

3. A female received intravenously, 10 cc. of the neutral red suspension and the urine was collected for eight and a half hours without any of the dye appearing in it. As above, it was present in the bile.

The two following experiments were carried out a year later than the former, with a different sample of neutral red. This could not all be removed by filtering and part of it even passed through a celloidin sac, so that it probably was partly in solution.

4. Ten cc. of filtered neutral red in sea-water was injected intravenously into an adult fish. Four hours later, examination showed the dye to be in both the bile and urine, but not in the stomach or spiral-valve contents.

5. Eight cc. of the dye in the above form was injected intravenously into an adult dogfish. There was 24 cc. of bright red urine collected in twenty-four hours, and at autopsy 2.5 cc of reddish-brown bile was removed from the gall-bladder. The 24 cc. of urine was diluted to 100 cc. with water and the 2.5 cc. of bile to 200 cc. Maximum redness was produced by neutralizing, and the color of the two were then of about the same intensity. Thus the total excretion of the dye by the liver was more than eighty times as much as that excreted by the kidney. The total amount of dye removed by the liver was not collected

because some escaped with bile into the intestine. The neutral red in the urine appeared to be in solution when examined under the microscope, whereas some, at least, of that in the bile was in granular form. Before the filtered neutral red was injected it appeared to have only very minute granules, smaller than those in the bile, so that in passing through the liver, granules must have collected together. Denis ('12) found the urine acid to litmus, but neutral red excreted in it retained its red color.

C. Congo red. The Congo red used could easily be removed by filtration or settling and would not pass through a celloidin membrane.

1. Two adult dogfish received intravenously each 5 cc. of a heavy suspension of Congo red in sea-water. After twelve hours the urine was still free from the dye, as was the stomach and spiral-valve contents. A considerable amount was present in the bile.

2. A young fish (0.25 kg.) received doses of Congo red of 12 cc. and 18 cc., respectively, on two successive days and was autopsied forty-eight hours after the first injection. The liver and spleen were stained deeply with the dye, but the other organs of the body cavity appeared practically normal in color. The gills were not examined. A large amount of the dye was present in the bile, a small amount in the spiral valve, and a trace in the stomach. The dye in the spiral valve entered with the bile probably entirely and that in the stomach probably was regurgitated from the spiral-valve.

D. Trypan blue. Trypan blue was used primarily in studying the excretory function. Its action as a vital stain, although not exhaustively studied, will be considered also from the data we have, because the reaction in selachians seems to be in some ways different from that in teleosts as described recently by Wislocki ('17). A brief review of our results with this dye has been published (Hoskins and Hoskins, '18).

1. An adult male was injected intravenously with 9 cc. of a ½ per cent suspension of filtered trypan blue in sea-water. Four hours later the animal received 6 cc. more. Fifteen hours later another injection of 6 cc. was made. Autopsy was performed

thirty hours after the first injection. The urine was examined several times and was free of dye until nearly the time of autopsy, when a trace of the red portion (Wislocki, '17) was present. The bile contained a considerable amount of the whole dye. It was also recovered from the serum, but not from the stomach or spiral-valve contents. The liver and spleen were of a slightly darker blue color than the other organs, but all of the organs as well as the skin and peritoneum appeared slightly colored by the dye.

Microscopical examinations were made of the digitiform gland, kidney, liver, spleen, spiral valve, stomach, skin, and musculature. All of these retained their blue color after fixation as seen in gross specimens, but blue granules are found under the microscope, definitely, in only the endothelium of the liver and spleen. The stain in the other organs and tissues is either too diffuse to be seen microscopically or else was all free in the blood-vessels, and hence washed out in preparing the sections.

2. A young fish (0.25 kg.) was given four daily intravenous injections of 3 cc. each of the above-mentioned trypan blue, and was autopsied five days after the first injection. The dye was found in considerable amount in the bile and serum and was also present in the spiral-valve, but not in the stomach contents. The liver and spleen were very dark blue in color as were the gills at the base of the filaments. The skin was dark blue before fixation, but light blue afterwards. The digitiform gland, spiral valve, post valvular intestine, peritoneum, and fascia of the muscles were light blue in color, the stomach was still lighter, and the kidney, except the peritoneal covering and connective tissue, was practically normal in color.

All these organs except the first three were of a lighter shade after fixation than before, owing doubtless to loss of blood which contained trypan blue.

Microscopical examination. a. Digitiform gland. Most of the dye is too diffuse to be located microscopically. A few leucocytes and endothelial cells of the capillaries of the parenchyma and serosa contain blue granules and one cell of a tubule contains eight such granules in a vacuole, but the amount of the dye detectable in this organ is too small to be of practical importance.

b. Kidney. Microscopically, the dye can be seen in only a few leucocytes and endothelial cells of the capillaries.

c. Liver. The liver contains most of the fixed trypan blue in the entire body. This organ is relatively large and in almost every cell in a large number of sections examined there are from five to twenty blue granules of different sizes (fig. 2). In places the granules appear to be in vacuoles. A rather large number of endothelial cells of the sinusoids likewise are seen to contain the dye. In figure 2 one such is seen nearly separated from the wall of the vessel and so full of stain that the nucleus is barely visible. In the liver of the dogfish there is a relatively large amount of normal pigment both in the sinusoids and in the parenchyma, collected into large masses. These black granules are often found in phagocytes. The large mass shown in a sinusoid in figure 2 is made up both of this black pigment and trypan blue granules. These black pigment masses may be found in the bile normally, and in the present experiment both black masses and masses of both blue and black granules were seen. There were found also in the bile many blue granules free and in escaped cells which are present also in normal bile. All the granules shown in figure 2 in the hepatic and endothelial cells and in the leucocyte are trypan blue. The mass at x is mostly normal black pigment.

d. Spleen. A free-hand section of the spleen showed the dye to be scattered uniformly through the tissue. The specimen was not examined microscopically, but nearly every cell must have ingested the dye.

e. The spiral valve, stomach, postvalvular portion of the intestine, muscles, and skin were still light blue in color after being embedded in paraffin, but under the microscope definite blue granules can be seen in only a few free phagocytes in the blood-vessels. A free-hand tangential section of the integument was cleared in oil, but the dye present was too diffuse to be made out after clearing. The pigment in the melanophores was all black and the subcutaneous vessels were colorless.

f. Gills. The sinuses which intervene between the arterial arches and the capillaries in the filaments, as described in the experiments with carmine, are lined with endothelium very phagocytic to trypan blue. The dye is in greater concentration in these cells than in any others examined. In some sinuses (figs. 3 and 4) every cell is engorged with the dye. Even in the large artery (fig. 3) a few of the endothelial cells contain blue granules. In figure 4 the cells and nuclei have rounded. Many are nearly detached or are lying free in the sinus ready to move with the blood stream as circulating phagocytes. Some of the granules in the cells seem to be contained in vacuoles, although the fact is not shown in the drawing. Most of the granules shown are trypan blue, but two cells marked '*x*' contain also some normal black pigment. In some cells the dye completely hides the nucleus. Practically all the sinuses examined are completely lined with cells filled with trypan blue, but a few sinuses are unstained.

A few cells in the capillaries contain blue granules. The epithelium covering the filaments does not contain trypan blue, although some investigators have suggested that the gills may excrete other substances besides gas.

3. *Excretory toxins*

A. Potassium chromate. 1. An adult dogfish received intramuscularly 150 mg. per kg. body weight of potassium chromate in a 1 per cent solution. Autopsy was performed after thirty hours and when the animal was moribund.

The kidneys and spiral valve were greatly congested, the latter containing a bloody fluid. Other organs appeared to be normal at autopsy.

2. An adult received intramuscularly 75 mg. per kg. body weight of potassium chromate in 1 per cent solution. Autopsy was performed after twenty-four hours while the animal was still active. The amount of toxin injected was less than that which Denis ('14) found to be necessary to kill a dogfish. In this specimen all the organs appeared normal except the spiral valve and kidney, which were slightly congested.

3. Four adults were injected intravenously with 100 mg. per kg. body weight of potassium chromate in dilute solution, to study the effect of sudden intoxication. Every one of the four died in a short time, varying from a few minutes to three hours, although they received much less toxin than is necessary to kill them if it were injected intramuscularly and slowly absorbed. In each of them at autopsy the blood was brown and 'granular' in appearance, showing that the erythrocytes had been destroyed and the haemoglobin oxidized by the chromate, which is a commonly used oxidizing reagent.

Microscopical examination. a. Digitiform gland. This organ in animal '1' is severely congested (fig. 10), but there is no great increase in the relative number of leucocytes. The central venous sinuses are greatly distended with blood. At the periphery most of the tubules have undergone vacuolar (hydropic) degeneration (figs. 11, 12, 13), which in some places is complete. Here the epithelial cells are cytolysed and the nuclei shrunken and darkly stained and in some cases are pyknotic. A few nuclei, on the other hand, are swollen and hypochromatic.

The area of extreme degeneration is about one-twelfth as wide as the radius of the gland. In a narrow strip, internal to this area, there is a gradual transition to normal condition, the cells exhibiting varying degrees of cytolysis and pyknosis or karyolysis. Scattered through the remainder of the gland are individual tubules showing mild cellular degeneration. It should be noted that in normal animals a few cells occasionally are degenerate, probably from cell inanition, (a term applied by Jackson ('16) in a study of the thyroid).

Cellular debris containing degenerated nuclei may be seen in some tubules and in the central lumen of the gland.

The erythrocytes in the digitiform artery appear normal, but in the central sinuses all appear granular and some are completely broken down.

2. The digitiform gland in the second animal was not injured by the potassium chromate.

b. Kidney. 1. The kidney in the first two animals is severely congested. Capillaries are dilated in the glomeruli and among the tubules. Erythrocytes show signs of degeneration. The glomeruli are not destroyed, but are probably somewhat injured. Many of their nuclei (fig. 6) are either hypochromatic or hyperchromatic and an occasional vacuole is seen in the capsule.

Two distinct types of degeneration are seen in the tubules of this specimen and a third type in the second animal. These two types we may call granular and hydropic (vacuolar), and of these, the former greatly predominates.

The granular degenerative process occurs as follows: First the cytoplasm loses its network appearance (fig. 7) in which the granules are very small and brightly stained with eosin. The granules become dull and adjacent cell walls disappear and finally the cytoplasm breaks through into the lumen. At the same time the entire tubule may shrink in places and pull away from its connective-tissue capsule. The degenerated mass of cytoplasm may spread and lose all shape (fig. 9). While these processes are occurring the nuclei are degenerating in the following manner. Most of them shrink, becoming hyperchromatic, and stain poorly. The chromatin may become merely a dull mass (fig. 8). The nuclei may shrink to nearly invisible size. In some tubules the nuclei degenerate differently. These gradually swell, becoming hypochromatic, and then undergo complete karyolysis. Both kinds may be seen in the same tubule.

The hydropic type of cellular degeneration is that in which vacuoles appear in the cells which gradually undergo cytolysis. The cytoplasm at first undergoes slight granular degeneration. In these cells the nuclei may undergo either hyperchromatosis or karyolysis. There is relatively little hydropic degeneration present in the chromate nephritis and it will be described later.

Destruction of epithelium is largely confined to the thick-walled secretory tubules of the posterior region of the kidney, where nearly every such tubule shows signs of degeneration.

There is an increase in the number of leucocytes present in the degenerated areas.

2. The kidney of the second animal was much less severely injured than in the first, as was to be expected since it received a smaller dose and was autopsied sooner

The glomeruli are seen to be very slightly injured, as is evidenced by their hyperchromatic and hypochromatic nuclei. A considerable number of secretory tubules show the beginning of either granular or hydropic degeneration. Animal '2' shows in 20 per cent of the tubules in the posterior part of the kidney a third type of nephritis that may be called hyaline. It is not seen in the kidney of the first animal. In this type there is a gradual accumulation in the cells of droplets of abright homogeneous substance which stains readily with eosin. In extreme cases the tubule is completely changed into a mere mass of these droplets. The nuclei may appear normal or hyperchromatic. The hyaline degeneration will be described later.

e. Liver. There is no serious degeneration in the first animal and almost none at all in the second. In the former a number of nuclei are hyperchromatic and in places the cytoplasm stains poorly. There is a slight congestion in both specimens. The ducts and gall-bladder are uninjured.

d. Spleen. The spleen was examined microscopically in the first animal only. It is congested, the capillaries and sinuses being somewhat dilated. They contain erythrocytes which appear to be degenerated. In these the nuclei are darkly stained and the haemoglobin appears granular. Some erythrocytes are entirely broken down and are shown only as nuclei surrounded by a few granules. The splenic cells proper and endothelium are intact but some stain poorly.

e. Spiral valve (figs. 14 and 15). The spiral valve shows similar changes in both animals, but the injury in the second is less severe than in the other.

Both are greatly congested and oedematous. The surface epithelium has completely desquamated in large patches, especially where it is most exposed, on the folds of the mucosa (fig. 15). The surface epithelium remaining stains poorly, appearnig dull, and the nuclei are either hypochromatic or hyperchromatic. A few cells are cytolysed. The epithelium in the bottom of the crypts is nearly normal in appearance. The erythrocytes in the spiral valve of the first animal show considerable injury, but those in the second do not.

f. Stomach. The stomach is not seriously injured in the first specimen and not at all in the second. In the former there is slight congestion, the surface epithelium contains a few hyperchromatic and a few hypochromatic nuclei and the deepest glands show slight cytolysis in a few cells.

g. Blood. As indicated above, severe injury to erythrocytes is done with potassium chromate. In some of the vessels of every organ examined there are degenerated red blood-cells, although in other vessels the cells are normal in appearance. The injury is greatest in congested areas where the flow of blood was interfered with. Intravenous injections of potassium chromate lakes the blood rather quickly.

B. Tartaric acid. An adult male received intramuscularly 100 mg. per kg. body weight of tartaric acid and was autopsied after thirty hours when it was moribund. All of the organs appeared normal except the spleen, which was pale.

Microscopical examination. a. Digitiform gland. This organ is severely congested, but except for a very slight cytolysis and hypochromatosis near the periphery there is evidently no injury to the tubules.

b. Kidney. Nephritis in all regions of the kidney is very severe. Congestion is marked. The glomeruli are not particularly affected, but have hyperchromatic nuclei. In some transverse sections of the posterior part of the kidney fully 90 per cent of the secretory tubules and many of the excretory tubules show signs of degeneration, ranging from mild changes to complete destruction (fig. 17).

All three types of nephritis are present, but the granular variety (fig. 19) predominates. Granular nephritis is much the same as that described in chromate nephritis (see figs. 8, 9, 18, 19, and 20). The destruction in the case of the tartaric acid nephritis is more complete (fig. 20), and in addition there is present in the surrounding tissue a very considerable number of cells with large eosinophilic granules and nuclei of different sizes. These granules stain more readily than the tubules. Some of these cells might possibly be degenerated erythrocytes, but most of them have nuclei resembling leucocytes and endothelial phagocytes. These cells were found only in this experiment. In places even the connective tissue and blood-vessels are injured.

Hydropic degeneration is confined largely to the thinner-walled (collecting) tubules, as may be seen from a careful study of figure 17. In some of these tubules the cell outline is fairly distinct and the nucleus nearly normal in appearance, but every bit of cytoplasm has dissolved out. In other tubules the nuclei are shrunken and darkly stained and the cell's walls are indistinguishable. Only a few of the secretory tubules contain vacuoles.

Hyaline degeneration is confined entirely to the secretory tubules. In this type the hyaline substance first appears in very small droplets near the nucleus. The small black dots in the cytoplasm in figure 17 are of this hyaline substance. The hyaline droplets gradually increase in size and number (fig. 21). They finally break through into the lumen of the tubule. During the process the nuclei may shrink and become hyperchromatic or swell and become hypochromatic, but they are not so severely injured as in granular degeneration.

c. Liver. The liver is not greatly congested. The tubules contain more debris than usual and both hypochromatic and hyperchromatic nuclei are rather numerous. In the sinuses are many cells with eosinophilic granules like those seen in the kidney, but in smaller number.

d. Spleen. The spleen itself is uninjured, but contains many of the eosinophilic cells noted above and also a considerable number of degenerate erythrocytes and free nuclei. There are also a few peculiarly shaped nuclei which will be described below.

e. Spiral valve. The injury done to the spiral valve by the tartaric acid is about as extensive as in the case of potassium chromate. Both karyorrhexis and pyknosis are shown in the epithelium. In the lamina propria of the mucosa there are numerous eosinophilic leucocytes. In sections of the spiral valve stained together with sections of a normal spiral valve the chromatin in the epithelial cells of the former which appear otherwise normal in structure stains much less darkly with haematoxylin than does that of the normal tissue.

g. Stomach. The stomach is in about the same condition as in the 'chromate' specimen. There are present in the blood-vessels and lamina propria numerous eosinophilic leucocytes.

C. Uranium nitrate. 1. An adult received intramuscularly 90 mg. per kg. body weight of uranium nitrate in 1 per cent solution. It lived forty-four hours and was then autopsied at once. The spiral valve was greatly congested, but the other organs appeared normal.

2. A male of 1 kg. received 100 mg. of uranium nitrate as above. Respiration ceased after two hours, but was restored by an hour of artificial respiration produced by inserting a tube of running water into the animal's mouth. The fish died after twenty-eight and a half hours and was immediately autopsied. The spiral valve contained bloody fluid and its mucosa was congested. A few small hemorrhages appeared in the kidney. No other abnormalities were noted.

3. An adult of 1 kg. received intramuscularly 100 mg. of uranium nitrate. A cannula was inserted into the bile duct to prevent any toxin that might be in the bile from reaching the spiral valve. After five hours the urine was tested and found to contain uranium nitrate. The next morning the animal received an additional 90 mg. of the toxin. At 8 P.M. it was still active, but died in the early morning, about forty hours after the first injection. The body was not yet rigid at 8 A.M., so tissues were fixed for microscopical examination, as few postmortem changes had had time to occur. This was confirmed by the microscopical examination. The kidneys were congested, but other organs appeared normal.

4. Eight dogfish of various sizes were injected with 100 mg. to 200 mg. per kg. body weight, of uranium nitrate. A cannula was inserted into the bile duct to protect the spiral valve from toxin in the bile. The urine was collected. Uranium nitrate

was found in the urine, but the color of the bile made it uncertain whether or not the toxin was present there. One animal receiving 200 mg. of the toxin in two equal daily doses survived for eighty hours, the others dying in shorter time. Two animals while still living were noticed to have a bloody discharge from the cloaca. Autopsy showed that in all the animals the spiral valve was greatly congested and its mucosa was injured. The stomach and the postvalvular portion of the intestine appeared to be uninjured.

Microscopical examination. Sections of the various organs of the first three animals described above, were examined microscopically.

a. Digitiform gland. In all specimens there is considerable congestion and slight cytolysis at the periphery and scattered through the gland, caused either directly by the toxin or by its interference with the blood supply, or from both causes. The injury was not very severe in any case.

b. Kidney. The kidney in every case is greatly congested, especially in the posterior region. The glomeruli are not very seriously injured in these specimens, but in most of them the capillaries are dilated and the nuclei of the capsule are hyperchromatic and shrunken.

Injury to the tubules is confined largely to the secretory tubules of the posterior portion of the gland (figs. 22, 23, 24, 25). In the third animal the changes are most severe, due either to the fact that it received more toxin than the first two animals, or else to postmortem changes, or to both reasons. In the third animal nearly every tubule in the posterior region is degenerated and some have completely disappeared. All three types of degeneration are seen, but the granular type predominates. Both hyperchromatization and hypochromatization of the nuclei are evident. In the former the nucleus gradually shrinks until it finally disappears (fig. 23). In the latter the nucleus absorbs water and swells often to two or three times the original diameter, the chromatin gradually disappearing until the nucleus looks like a ring. In the third specimen almost every nucleus has changed from the normal oval to the spherical shape, and most of them have suffered chromatin changes. The blood-vessels in the kidney of this animal contain a large amount of debris.

In the first animal there are present in the blood-vessels of the degenerated region of the kidney many nuclei of a very peculiar shape (fig. 26). This may indicate an attempt at amitosis by phagocytes, probably in the spleen and gills. One cannot make out any cytoplasm, so that if a cell wall is present it is tightly adherent to the nucleus. On the whole, the kidney is less seriously injured in the uranium series than in the chromate or tartaric. However, the size of the dose of the toxin and the time element are probably the most important factors in the amount of injury produced.

In the anterior region the tubules show no signs of degeneration in some sections, and very few in any. The congestion is mild as compared with that in the posterior region. A few tubules stain poorly and their cytoplasm is quite granular.

e. Liver. The liver and gall-bladder are practically uninjured even in the third animal. In all sections a few nuclei of the hepatic cells and ducts are hyperchromatic and shrunken, but not in more than 12 per cent at most. In the third animal (which received a large dose and which died before autopsy) the ducts contain considerable debris, and a few of their cells are very slightly cytolysed.

d. Spleen. The spleen is congested in every specimen. The sinuses are dilated and contain granular debris, which probably has come in from the kidney. The endothelial cells of the sinuses contain these granules and their nuclei are often swollen and hypochromatic. In the first animal (fig. 27) there are many peculiar-shaped nuclei which resemble those seen in the kidney and gills. Some of these at least are endothelial in origin, as they can be seen in the cells lining the sinuses. They may represent abnormal amitosis. Many mitotic figures can be seen in the spleen of this animal, although they are fewer than when carmine was injected. The splenic cells proper are not particularly abnormal in appearance, although a few have hypochromatic nuclei.

e. Spiral-valve. In the first two animals in which the bile (presumably containing the toxin) was allowed to enter the gut, the spiral valve showed approximately the same amount of injury as in the chromate and tartaric experiments. In the third animal in which the bile was kept out of the intestine the injury is less extensive than in the others, although there is some congestion of the mucosa and desquamation of its surface epithelium. This was true also of other animals which were not examined microscopically, as stated above (4).

f. Stomach. The stomach in the first two animals resembles that in the chromate experiments. In the third, the stomach is practically normal, and the very few alterations observed might be postmortem in origin, or the result of vascular injury which causes cell inanition.

g. Postvalvular intestine. That portion of the intestine posterior to the spiral valve was examined microscopically in the second animal, and is entirely normal in appearance, although the spiral valve which empties into it is seriously injured. Its epithelium is stratified while that of the spiral valve is simple columnar.

h. Gills. The gills of the first animal were examined and show some signs of injury. In the sinuses at the base of the filaments the endothelial cells have rounded as they do when granules are injected. Here are seen many peculiar nuclei (fig. 27) such as were seen in the spleen and kidney, some of which may be found protruding from the endothelium. The endothelial cells in places are full of dull granules which have come originally from the blood, or else are formed in the cells by degeneration. Many free cells are to be found in the sinuses, indicating a proliferation of the endothelium.

DISCUSSION

1. Digitiform gland

The digitiform gland reacted somewhat as an excretory organ, but it is not very efficient as such. In this it resembles the kidney. Its normal secretion collected from three adult dogfish averaged only 0.25 cc. in six hours, or at the rate of 1 cc. a day. The excretion is alkaline to litmus. It is clear, but contains bits of mucus. The amount of this excretion is not copious, but the entire amount produced by both kidneys is only a little over 20 cc. in twenty-four hours. Since the size of the digitiform gland is much less than one-twentieth of that of the kidneys, its excretion is relatively greater than the amount of urine given off. Crawford ('99) obtained urea from the digitiform gland and suggested the possibility of excretory function for this organ. Hyrtl ('58) regarded it as an accessory sex gland, and Blanchard ('82) stated that it is potentially able to aid digestion because an extract of it splits starch and emulsifies fat. An extract is, however, not the same thing as a secretion and hence Blanchard's conclusion may not be correct. It should be borne in mind that the product from the gland is discharged into the intestine just above the cloaca at a place where digestion has doubtless ceased. Morgera ('16) thought that the digitiform gland has an internal secretion which causes the intestines to contract, thus aiding in the passage of substances through the gut, because he found that passage of food through the intestine was checked by extirpation of the gland and restored by the injection of an extract of it. One must consider that the interference with the passage of food in these experiments may have been but a temporary disturbance from the operation, that would have disappeared later. Also, as stated above a glandular extract is not the same thing as a normal secretion of the gland.

Cushny ('17) believes that urine is excreted by glomeruli and not by the renal tubules. If the secretion of the digitiform gland is a true excretion, then the renal tubules may also be able to excrete, in which case, Cushny's theory is incorrect.

We were able to recover in the excretion from the digitiform gland after injection into the animal two solutions, namely, potassium iodide and pthalein, but not any of the others used. The other solutions if present were too dilute to be demonstrated. Weed's solution passed into the cells of the tubules, as shown microscopically, only in small amounts, although it was present in the blood-vessels in relatively concentrated form.

Particulate matter is not taken into the tubules of the digitiform gland, except for an occasional granule, even though the particles are very small. In this the gland resembles the kidney, so the failure to take in granules does not argue against possible excretory function.

Excretory toxins caused congestion in all cases, but in only one animal receiving potassium chromate was the injury to the tubules very severe. Morgera ('16) obtained cytolysis of the tubules by cutting off the blood supply, and the congestion noted in our animals may account in this way for some of the degeneration noticed. One finds occasional cytolysed cells in the digitiform gland of normal animals. The question of cellular inanition must be considered in all experimental degeneration of tissues produced by administered toxins. The toxins we used certainly were injurious to erythrocytes, and this together with interference with blood supply in congested organs must deprive the cells of needed substances. However, we believe that a part of the injury seen was caused by the toxins which the gland was trying to excrete.

2. Kidney

The kidney (mesonephros) of the dogfish is of far less importance to the animal than the kidney (metanephros) of mammals, as has been noted previously. Denis ('12) found that adults produce an average of only 21.7 cc. of urine a day, and in our experiments the greatest amount of urine obtained was 24 cc. in twenty four hours, from an animal weighing more than 1.5 kg. The urea in the urine amounts to 0.6 to 1.4 per cent, or less than half the concentration of that in the blood (Baglioni, '06) and less than half that in mammalian urine. This difference is made up partly at least by a considerable amount of urea excreted in the bile. The great concentration of urea in the blood seems to be necessary to preserve life, for Baglioni ('05) found that isotonic (3.5 per cent) salt solution without urea was unsatisfactory for perfusion experiments. This requirement does not account for the small amount of urea excreted, as has been argued, because, an equilibrium having been established, all urea formed would need to be eliminated. Hence if there is little urea excreted it is because relatively little is formed. The limited excretion of urea in the urine is dependent upon the metabolism, which must proceed at a slow rate in these forms, and also upon the amount of urea excreted in the bile.

MacCallum, in the 1917 Herter Lectures in New York, stated that the selachians entered the sea when its salt concentration was less than it now is and that as the sea became concentrated the selachians retained more and more urea in the blood to keep it isotonic with the sea-water.

We found that the dogfish kidney is able to excrete injected solutions quite as rapidly as the mammalian kidney and hence the inefficiency of the former is not one of rate of excretion, but of amount and of inability to eliminate certain kinds of substance. Non-toxic solutions of all kinds when injected into the muscles of the body wall appeared in the urine in fourteen or fifteen minutes, and when injected intravenously appeared in the urine in about eight minutes. In this rate the dogfish compares favorably with mammals (Rowntree and Geraghty, '12). The dogfish kidney would probably eliminate all of an injected solution in time, if it were not for the fact that the liver quickly eliminates the larger part of it. The liver is many times larger than the kidneys and has taken over much of the excretory function. As noted above, when pheno-sulphonepthalein was injected, the liver excreted in a given time more than twice as much of it as did the kidneys, and in concentration many times greater. However, the difference in amount excreted by the two organs was less than the relative difference in size by which the liver surpasses the kidney.

Colored solutions stained the posterior part of the kidney more intensely than the anterior end, thus demonstrating the fact that in the former the excretory function is better developed than in the anterior or sexual (Felix, '12) end. The anterior region is able to excrete some part of solutions injected, so it is not entirely devoid of excretory function, as some investigators have believed. This point should be studied further. The ability of the urine to reduce copper sulphate after sucrose had been injected demonstrates that the selachians can invert this sugar as mammals do (Kuryama, '16).

The dogfish kidney was not stained vitally by injected suspensions of various dyes, since they were not removed from the blood by the kidney, at least up to five days' time, even when administered in very considerable amounts. There was no difference in this respect between the reaction to very minute particles (trypan blue) or coarse granules (powdered carmine). One sample of neutral red passed through the kidney and one did not do so. The first probably formed a true solution. Attention of investigators should be called to the fact that some differences they obtain in the use of vital dyes in experiments may well be due to differences in the manufacture of these substances. Colloidal dyes, if impure, may be soluble.

The inability of the kidney of dogfish to remove particulate matter from the blood is another difference between this organ and the kidney of higher animals, as shown in the very numerous experiments of Chrzonszczewski ('64), Siebel ('86), Schmidt ('91), Muscatello ('95), Buxton and Torrey ('06), Suzuki ('12), Höber ('14), Kiyono ('14), Evans-Schuleman ('14), Downey ('17), and many others.

In higher animals the renal epithelium and sometimes the cavity of the glomerulus may be seen to contain particles that have been injected, but in the dogfish, if such matter is present in the kidney parenchyma or glomeruli, it is much too diffuse to be detected in our preparations. The only dye which really stained the kidneys in our animals was indigo-carmine, and this appeared to be in solution. Fresh kidney tissue was not examined microscopically in the trypan blue experiments, but it probably contained the pink substance that Wislocki noted in teleosts, as the urine was pink.

The endothelium of the renal capillaries in the dogfish is but poorly able to ingest particulate matter from the blood as compared with that of the renal portal vessels of teleosts, as described by Wislocki ('17). Wislocki found that this endothelium becomes blue from ingested dye after injections of trypan blue, while in our animals up to five days only an occasional one of these cells was able to take in visible amounts of carmine or trypan blue. It is possib'e that after a greater length of time this endothelium in dogfish would react as does that of teleosts toward trypan blue. . Wislocki also found a small amount of dye in the epithelium of the kidney in teleosts. This he believed might have been resorbed from the urine. Previous investigators have agreed with him (Cushny, '17). He also implies that the endothelium of the vessels in the kidney developed from the aorta is not able to take in and store trypan blue, but it is difficult in microscopical sections to distinguish the efferent glomerular vessels from renal-portal vessels.

Wislocki suggests three possible functions of the renal-portal endothelium in the kidney which he calls a mechanism. The first function is to prevent loss of colloidal metabolites, thus accounting for the relatively low output of nitrogen in fish urine, but the real reason for this is probably in the slow rate of metabolism in fish and a large output of nitrogen in the bile. The second function Wislocki attributes to the renal-portal endothelium is to protect the animal from toxic substances, but in an animal not being experimented on, any toxic substances present would more likely be in solution than in suspension, and solutions pass freely through the kidney. The third function referred to is a resorption of substances from the urine, but the reaction to trypan blue does not prove any such function, as it has not been shown that in these experiments the endothelium in question absorbed dye in this way. The possibility of such resorption of dyes has been considered previously, as noted above. All that Wislocki's experiments actually prove is that the endothelium of the renal vessels is phagocytic, but not very permeable, if permeable at all, to particulate matter.

The liver excretes particles freely, but whether such function failed to develop in the kidney because of this fact or whether the inability of the kidney to excrete particulate matter forced the liver to develop the function cannot be determined. Normally, particles in the circulation which are to be eliminated are mostly bile pigments which of course need not go into the urine.

The effects of excretory toxins on the kidney in dogfish are somewhat the same as in mammals, as described by Ophuls ('08, '11), Schlayer-Takayasu ('09), Suzuki ('12), Pohl ('12), Dickson ('12), Christian-O'Hare ('13), Underhill-Blatherwick ('14), Potter-Bell ('15), Tribe-Hopkins-Barcroft (16), and various others who administered potassium chromate, tartaric acid, and uranium nitrate. Denis ('13) noted that dogfish can withstand many times as much potassium chromate and uranium nitrate as will kill a mammal, and we can add tartaric acid to the list. One of our fish which received 200 mg. per kg. body weight of uranium nitrate lived for eighty hours. In extreme cases the dogfish kidney shows nearly complete destruction of the tubules by these poisons. The blood-vessels usually are not noticeably injured, but severe congestion occurs. The glomeruli, as in mammals, are injured only slightly when at all. Three distinct types of degenerative processes in the tubules are produced by all three of the toxins used. The deposit of hyaline substance in renal tubules in mammals after injection of tartaric acid was noted by Underhill-Blatherwick, and Christian-O'Hare found it in blood-vessels after uranium poisoning. Granular degeneration is probably a constant type in all such experiments, and hydropic degeneration usually but not always occurs with it. In our specimens there is some vacuolization in the cells showing the former type of degeneration, but some cells undergo cytolysis without first passing through granular change. The reason for these three different kinds of degeneration is not apparent. All three types were present in the same animal in some of the experiments and often two could be seen in a single tubule. They are not simply different steps in one and the same process as a study of the accompanying figures easily shows. In the hyaline type a definite new substance is formed. There are two distinct types of nuclear degeneration in our experiments with nephritis and they are exactly opposite in nature. In one type the nucleus absorbs water, swells to two or three times its normal size, and the nuclear substances gradually go into solution, while in the other type the nucleus becomes opaque and shrinks until it completely disappears. This shrinkage is gradual at times and the nucleus retains a regular outline, but in other cases the loss of karyoplasm is sudden and the nuclear membrane collapses and is then irregular. Both types of nuclear degeneration are found in all the specimens examined and both may occur in the same tubule.

The cellular changes which one observes in nephritis are doubtless due to cellular inanition as well as to poisoning of the cells. Jackson ('16) has described some of these changes in the thyroid of starved rats. Congestion of the organs and injury or destruction of erythrocytes interferes with the general metabolism of the cells, thus bringing about inanition changes.

Differences in the process of degeneration in different cells and nuclei in nephritic kidneys may be influenced by their sudden or their slow death.

3. *Liver*

The liver of selachians and probably of other fish is a very important excretory organ, although Pillet ('90) stated that its biliary function is not well developed. Denis ('13) stated that the liver of dogfish apparently has an excretory function, but did not give any reasons for the belief. The liver, like the other organs of selachii contains a large amount of urea (Staedler-Frerichs, '58). v. Schroeder ('90) stated that this amounts to 1.36 per cent of the entire liver substance. Heidenhain ('83) found urea in the bile of selachians, Hammersten ('97) stated that it occurs there in large amounts, and Van Slyke-White ('11) determined the concentration of urea in the bile to be 1.7 per cent. This is greater than that in the urine (Baglioni, '06), and more than half as great as that in the blood (2.6 per cent, v. Schroeder, '90).

All the non-toxic solutions we injected were recovered in the bile. A quantitative determination of the relative amount of an injected solution (pthalein) excreted by the liver showed that there was present in the gall-bladder five and a half hours after injection three times as much pthalein as had been excreted in the urine. Some pthalein had escaped with bile into the intestine and some was still present in the bile ducts. In mammals the kidneys normally excrete nearly all of such injected pthalein in four hours and very little appears in the bile at all (Rowntree and Geraghty, '12). In mammals with tartrate nephritis, or with their renal veins ligated, injected pthalein is excreted by the liver (Underhill-Blatherwick, '14).

All of the injected granular dyes appeared in the bile in considerable concentration, and were not excreted by any organ other than the liver, so far as determined. Siebel ('86) believed that frogs and dogs receiving injections of carmine excreted such particles through the liver, lungs, and tonsils, and Kiyono ('14) and numerous other investigators have found that mammals excrete lithium carmine through the kidney.

In our experiments one sample of neutral red appeared after injection in both the urine and bile, but its concentration in the latter was more than eighty times as great as in the former. It is believed that some of this neutral red was in solution and that it was this part which passed through the kidney. The liver was able to excrete the dye both in the dissolved and in the granular form. Another sample of neutral red was excreted only in the bile.

Trypan blue after injection into dogfish collects in the hepatic parenchyma and the cells lining the sinusoids. Wislocki ('17) noted this also. It is excreted freely into the bile. In bony fishes Wislocki found that trypan blue entered the bile, but was not stored in the liver cells. He thought that it diffused directly into the bile ducts from the blood-vessels but more probably it passed through the liver cells too diffusely to be seen under the microscope.

In the dogfish there is present normally in the liver and bile a very large amount of bile pigment (Pillet, '90) and after injection of trypan blue some of the masses of pigment seen in sections of the liver contain both trypan blue granules and normal pigment mixed together. These masses of mixed pigment may be seen in the bile also, thus showing that the excretion of particulate matter by the liver is a normal function and is not due merely to experimental conditions. Experimenters are often inclined to overlook the fact that results they obtain do not necessarily portray normal functions, because their experiments place their animals under abnormal conditions.

Excretory toxins usually caused congestion of the liver, but not serious injury to the parenchyma. We did not prove absolutely that the toxins passed through the liver into the bile, but we believe that they did so because all the other solutions and suspensions were recovered in the bile, and because the liver itself was injured by the toxins. Tests were made for uranium nitrate, but the green color of the bile prevented our determining whether the injected toxin was present or not. Injury to the liver was indicated by relatively few hyperchromatic shrunken nuclei and an increased amount of granular débris in the bile ducts. In none of the sections examined did more than 12 per cent of the nuclei appear injured. If the liver is able to eliminate excretory toxins, as it probably does, and without serious injury to itself, we must agree with Denis ('13) that this helps explain why dogfish are able to withstand large doses of such poisons.

4. Spleen

The spleen in the experiments with Weed's solution was seen to be impregnated with Prussian blue granules. This indicates that the vessels open directly into the pulp or else that the endothelium is very permeable to solutions because relatively more Prussian blue was found in this organ than in other non-excretory organs. The spleen of the dogfish seems to be very well adapted for phagocytic activity, although Wislocki ('17) believes that such is not the case in bony fishes.

Injected granules of dyes are taken up very quickly by the spleen which thus becomes deeply stained. In this respect it is equaled only by the liver. Injection of particulate matter was followed by a leucocytosis, and the number of mitotic figures seen in such cases is greatly increased.

Toxins caused congestion in the spleen in most specimens. In one uranium nitrate experiment there were present in the spleen a very large number of peculiar-shaped nuclei. These are found only occasionally in the other specimens. They probably indicate an abnormal attempt at leucocytosis by amitotic division.

Other masses of lymphoid tissue were not examined, but they probably are phagocytic to dye granules.

5. Spiral valve and postvalvular intestine

Denis ('13) mentions the possibility of an excretory function possessed by the intestine of dogfish. Several of the solutions and dyes which we injected were found later in the spiral valve. However, these entered the intestine partly if not entirely in the bile. With Weed's solution Prussian blue was not deposited in the epithelium nor were any granules of injected dye found in it. Potassium iodide was obtained in the spiral valve after nijection, but this will go through almost any gland and is not a specific test. Indigo-carmine did not stain the epithelium, nor could it be detected in the contents of the spiral valve. Experiments in which solutions of various kinds, including dyes, are injected, should be performed with dogfish in which bile is prevented from entering the intestine. We hope to do this at a later date. The toxins injected caused severe injury to the spiral valve whether the bile was permitted to enter the intestine or not. In every case there was severe congestion and destruction of a considerable amount of the epithelium. The capillaries in the mucosa ruptured producing hemorrhage into the intestine. These vessels were injured probably more that those in any other organ, although there was some vascular injury in the kidney. The spiral-valve contents were tested for the toxins that had been injected, but even with small amounts of blood and bile present the tests were not sufficient to determine the presence of the toxins. Probably more delicate tests would be successful. Our not finding some of the injected solutions in the spiral-valve contents also was due possibly to tests not sufficiently delicate. While the injury of the spiral valve by excretory toxins does not absolutely prove that this organ has normally an excretory function, it tends to support the theory. The fact that intestine posterior to the spiral valve was not injured at all by the injected poisons adds evidence in favor of this theory.

6. *Stomach*

A few solutions and one granular dye were recovered in the stomach, but the possibility of regurgitated bile was not eliminated as should have been done. Weed's Prussian blue was not deposited in the epithelium of the stomach nor were any of the injected dyes. The excretory toxins caused slight congestion of the stomach in some cases and in a few specimens the surface epithelium stained poorly. A small amount of cytolysis was present in some cases, but the slight damage noted in the stomach might have been done by toxin regurgitated from the intestine or by cell inanition resulting from vascular injury. Regurgitation is often observed.

Cecil-Weil ('17) found that after injection of Congo-red into patients with gastric ulcers it could be recovered in the stomach contents, but they admitted the fact that it is excreted in the bile and they did not eliminate the possibility of regurgitation of bile into the stomach. The stomach in selachians apparently is able to excrete only a few injected solutions, and is probably not normally an excretory organ of any importance, if it has this function at all.

7. *Gills*

In the experiments with non-toxic solutions the gills were examined in only those animals receiving Weed's solution. In these specimens Prussian blue was found in the blood-vessels of course, but there was none in the epithelium covering the gills nor in the connective tissue. This argues against the theory that the gills can excrete solutions. Since the gills are bathed by the isotonic sea-water, it would seem natural that solutions might diffuse into it from the branchial vessels, but we have no evidence that this occurs.

Granular dyes are ingested freely by the endothelium which lines the sinuses that are interposed between the arterial arches and the capillaries in the gill filaments. It is also ingested by the endothelium of the branchial arteries. In fact, in these cells of the gill sinuses the phagocytic function seems to be more highly developed than in any other cells in the entire body. They are described in a previous section (see 'Experiments'). These cells lining the sinuses are one of the sources of free phagocytes which circulate through the body. Wislocki ('17) does not mention these cells in his discussion of endothelial phagocytes in fishes. He states that in no endothelium in fishes has trypan blue been seen except in the lymphatics, renal- and hepatic-portal vessels, and splenic sinuses. He apparently would use trypan blue to distinguish between blood-vascular endothelium (other than 'specialized' endothelium of the kidney, spleen, and liver) and lymphatic endothelium. He also would use trypan blue to solve the problem of the origin of lymphatics in fishes. We do not believe that trypan blue or other particulate substances can be used in this way. It is highly probable that more extensive research would disclose the fact that the endothelium in various blood-vessels, both arterial and venous, in different parts of the fish's body, is able to ingest trypan blue. The rate at which the dye moves through the vessels is probably one of the most important factors in this, as is believed by Downey ('17). This could be tested by decreasing the rate of blood flow in different vessels with ligatures or other means. Downey ('17) stopped the blood flow completely and found that the leucocytes could then ingest dye granules. In an attempt to distinguish between lymphatic and blood-vascular endothelium from the ingestion of trypan blue, one should not set aside all blood-vascular endothelium that is phagocytic to the dye as 'specialized endothelium.'

Cells in general react toward trypan blue as they do toward other small particles in the circulation and it is not to be used as a specific cure-all for our angiological troubles. Arey ('17) and Downey ('17) have pointed out several ways in which the use of trypan blue has been overdone.

In our experiments the sinuses in the gills were affected by toxins in somewhat the same way as those in the spleen. There was a proliferation of the endothelium to produce free phagocytes. Some of these cells stain poorly and in some the cytoplasm is coarsely granular. There are present in one specimen poisoned with uranium nitrate, peculiarly shaped nuclei like those found in the spleen which probably indicate an abnormal amitosis.

8. *Body wall (muscles, skin, and peritoneum)*

In these tissues dyes were recovered only in the blood-vessels and connective tissue where an occasional phagocyte is seen. These tissues were not injured by the toxins so far as could be determined.

9. *Vascular system*

This system has already been discussed in part. Leucocytes and fixed phagocytes of endothelial and tissue origin were seen to contain trypan blue and carmine granules. Carmine was found ingested by endothelial cells lining the heart. Fixed phagocytes in the spleen, kidney, and gills apparently are injured by circulating toxins. Phagocytes containing carmine were found in the circulating blood within two hours after injection of the dye.

The blood-vessels of all the organs examined were congested by the excretory toxins injected, the most severe congestion occurring in the digitiform gland, kidney, and spiral valve. All three toxins—potassium chromate, tartaric acid, and uranium nitrate—caused injury to the erythrocytes, and when potassium chromate was injected directly into the blood-vessels these cells were destroyed, sometimes in a few minutes. Ophuls ('11) stated, in his paper on potassium chromate nephritis in mammals, that the cause of death in such experiments is not due to the injury to the kidney. It is probable that death is due to injury to the blood corpuscles, especially the erythrocytes. In all our sections of tissues, from animals that had received injections of excretory toxins, degenerated erythrocytes can be seen. When we injected potassium chromate intravascularly, the blood was laked and the hemoglobin oxidized, as shown by the 'granular' appearance and brown color of the blood at autopsy. Lymphatics were not stained by trypan blue or carmine to any noticeable extent up to the end of five days' time, although they might take up these dyes if more time were allowed.

SUMMARY AND CONCLUSIONS

1. *Digitiform gland*

The digitiform gland of Mustelus excretes about 1 cc. a day of clear alkaline fluid containing urea. Certain injected solutions, but no suspensions, were recovered in this excretion. Injections of excretory toxins injure this gland.

2. *Kidney (mesonephros)*

The selachian kidney is a less efficient excretory organ than the mammalian kidney (metanephros). The daily urine output amounts to about 18 cc. per kg. body weight, with a urea content of about 1 per cent (Baglioni, '06). The dogfish kidney excretes injected solutions as rapidly as the mammalian kidney, but only in small amounts, the solutions appearing in the urine in eight minutes after intravenous injection and in fourteen minutes after intramuscular injection. The dogfish kidney does not excrete injected particulate matter and the tubules are not noticeably stained by insoluble vital dyes, at least in five days. Large amounts of renal toxins (potassium chromate, tartaric acid, and uranium nitrate) are withstood by the dogfish for long periods. These toxins produce nephritis in which three different types of degeneration occur. These are hyaline, granular, and hydropic degeneration of the cytoplasm, especially of the excretory tubules. The nuclei are destroyed after either hyperchromatosis or hypochromatosis. Some of these cellular changes are due to inanition. The blood-vessels in the glomeruli and among the tubules are not seriously affected except after severe necrosis. The capsules of the glomeruli are only slightly injured.

3. *Liver*

The liver in dogfish is relatively a very efficient excretory organ. The bile contains 1.7 per cent of urea (Van Slyke-White, '11). The liver eliminates injected solutions and suspensions readily and in great concentration. In a given time the liver excretes injected solutions in several times the amount excreted by the kidney. The liver is the only organ in the body definitely proven in our experiments as able to excrete particulate matter. It is stained deeply by such substances. The liver contains a great deal of normal pigment which is excreted in the bile in the same way that injected pigments are excreted, thus demonstrating that the latter act is a normal process.

Excretory toxins are probably excreted by the liver and they cause very little injury to this organ. When the kidneys were almost entirely destroyed, the liver showed slight injury to about 12 per cent of the nuclei, which were hyperchromatic and slightly shrunken, and in the bile ducts there was more than the usual amount of granular substance, probably degenerated cytoplasm. The vessels of the liver are discussed below.

4. Spleen

The spleen is very well adapted for phagocytic activity, and is stained deeply by injected insoluble dyes. Injected particles produce leucocytosis. Toxins cause congestion in the spleen, the endothelium appears to be injured, very peculiarly shaped nuclei may be produced, and many nuclei become hypochromatic.

5. Spiral valve

The spiral valve has previously been considered to possess excretory function and may have such function for certain dissolved substances, but not all solutions we injected could be recovered in the intestine and none of the dyes were taken up by the epithelium. Excretory toxins pass through this epithelium and destroy it, although they do not injure the intestine immediately anterior or posterior to the spiral valve. We believe with Denis ('13) that the spiral valve has some excretory function.

6. Stomach

A few injected solutions were recovered from the stomach, but we do not believe that this organ has an excretory function. Excretory toxins do not injure it directly and vital dyes do not stain its epithelium.

7. *Gills*

The endothelium lining the arterial arches and especially the large sinuses in the gills is very phagocytic to injected trypan blue and coarsely powdered carmine. It stores such substances in concentration greater than that in any other cells examined, and at the same time it proliferates and produces free phagocytes which circulate through the body. Such proliferation and an abnormal amitosis occur after injection of excretory toxins, which also seem to produce degeneration of these cells. There is no indication that the gills are able to excrete solutions or particulate matter into the water surrounding them. They are probably a normal source of phagocytes for the circulation.

8. *Body wall*

The muscles, skin, and peritoneum had in general a negative reaction to non-toxic solutions, vital dyes, and toxins.

9. *Vascular system*

The blood-vascular system reacted negatively to non-toxic solutions so far as determined, except that sucrose in the blood was inverted. Trypan blue and carmine were found in occasional leucocytes and endothelial cells in the blood-vessels of the kidney, digitiform gland, spiral valve, stomach, and body wall. Endothelium lining the branchial and splenic sinuses and hepatic sinusoids is very phagocytic toward trypan blue and carmine. The arterial arches of the gills are lined with endothelium phagocytic to trypan blue.

The lymphatics were not noticeably stained with vital dyes up to five days, but the subject needs further study.

Excretory toxins cause slight injury to the fixed endothelial phagocytes, especially in the spleen and gills. They cause congestion in all the organs especially in the kidney, digitiform gland, and spiral valve. If injected intramuscularly they cause slow degeneration of the erythrocytes, and if injected intravenously, sudden destruction of these cells. This destruction is probably the cause of death in some cases of experimental nephritis studied by other investigators.

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PLATES

PLATE I

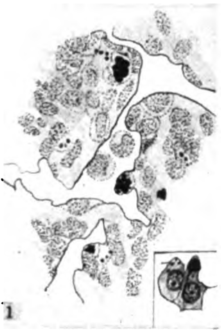
EXPLANATION OF FIGURES

VITAL DYES

1 Spleen four days after injection of coarse carmine granules. The black bodies are carmine. Insert shows an endothelial cell with a pseudopodial process full of carmine.

2. Liver five days after injection of trypan blue. Black bodies are trypan blue except in the cell at x which contains both trypan blue and normal black pigment.

3 to 5 Gill five days after injection of trypan blue. Figure 3 shows arterial arch and sinuses at the base of the filaments lined with endothelium which has ingested trypan blue, and containing free phagocytes filled with the dye; figure 4 shows normal endothelium from a sinus, and figure 5 shows two sinuses from figure 3, showing the reaction to the injected dye. The phagocyte at x contains both blue and black granules.



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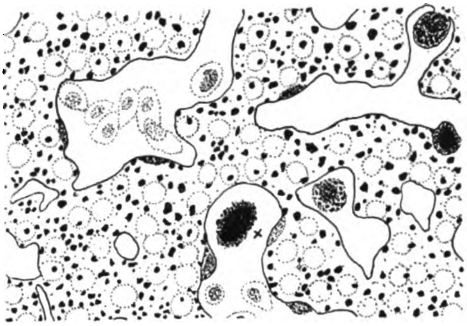

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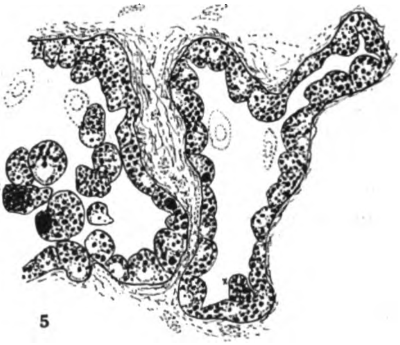

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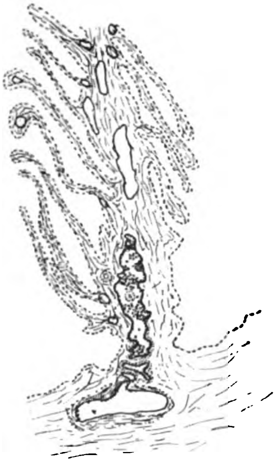

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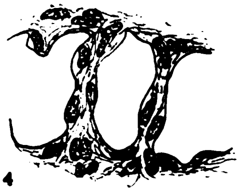

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PLATE 2

EXPLANATION OF FIGURES

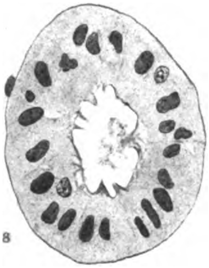
KIDNEY. POTASSIUM CHROMATE

6 Normal excretory tubule.

7 Glomerulus and collecting tubule after poisoning. Note the shrunken nuclei and vacuoles.

8 Excretory tubule. Note the granular degeneration and shrunken nuclei.

9 Extreme case of granular degeneration. The supporting connective tissue has broken and allowed the débris to spread. Desquamated cells become round. Hyaline and hydropic degeneration not shown.



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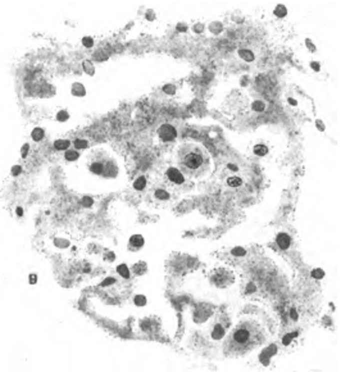

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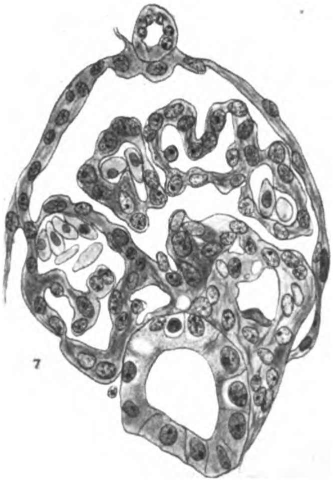

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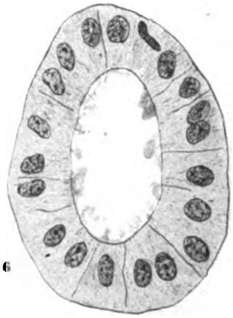

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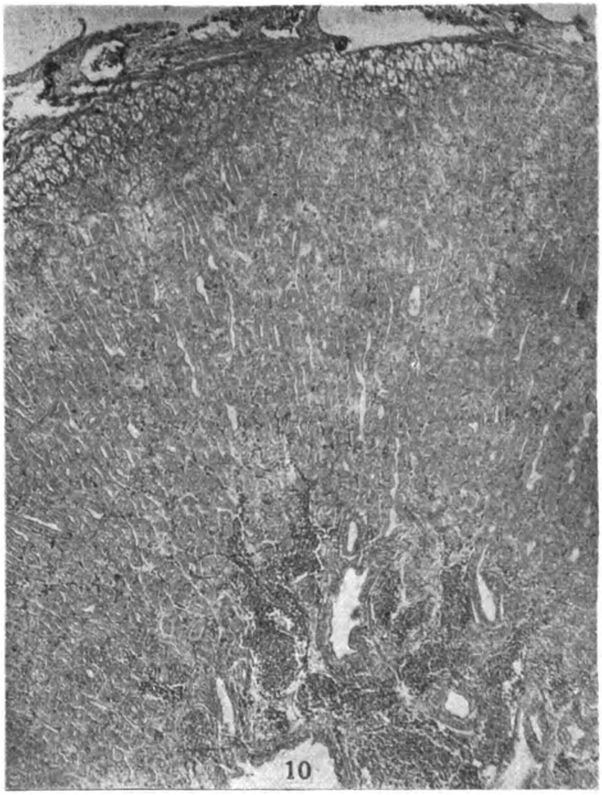
PLATE 3

EXPLANATION OF FIGURES

DIGITIFORM GLAND. POTASSIUM CHROMATE

10 Shows congestion at the center and cytolysis at the periphery.
11 Shows a normal tubule.
12 and 13 Show different degrees of cytolysis with shrunken, opaque nuclei.



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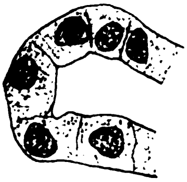

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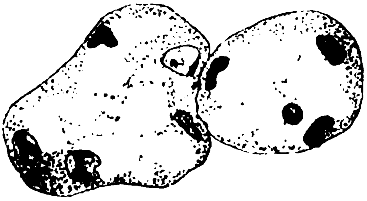

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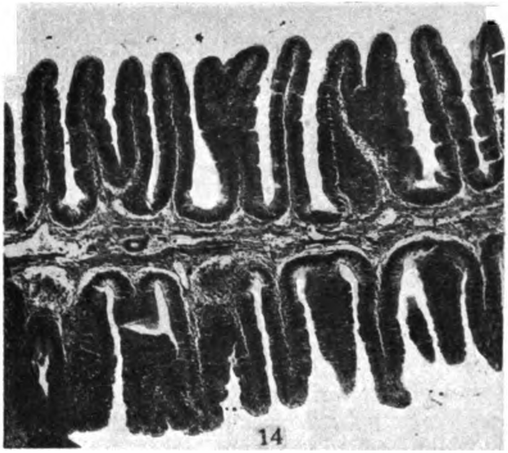
PLATE 4

EXPLANATION OF FIGURES

SPIRAL VALVE. POTASSIUM CHROMATE

14 Normal spiral valve.

15 Effect on spiral valve of potassium chromate poisoning. Note the loss of innermost epithelium. Severe congestion. The entire section is poorly stained, although it was prepared similarly and stained simultaneously with the section shown in figure 14.



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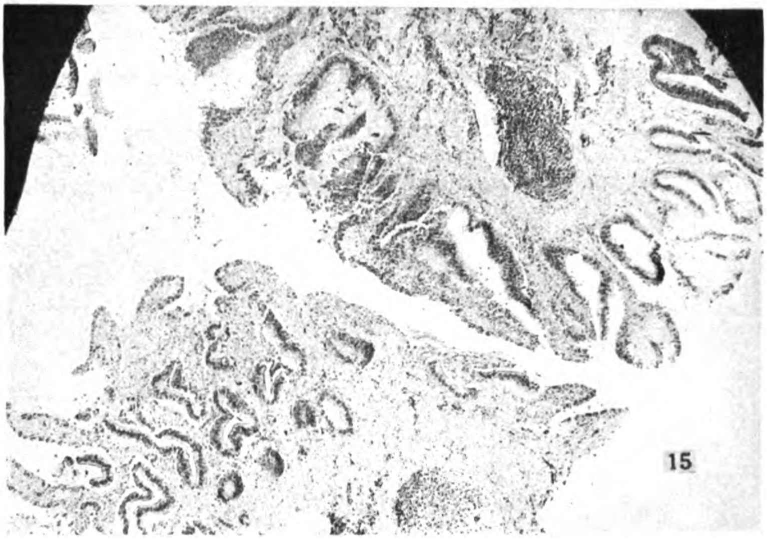

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PLATE 5

EXPLANATION OF FIGURES

KIDNEY. TARTARIC ACID

16 Normal kidney.

17 Kidney poisoned with tartaric acid. Note the shrunken nuclei, broken tubules, and débris. The very small black bodies in the cells of the excretory tubules are of eosinophilic hyaline substance.

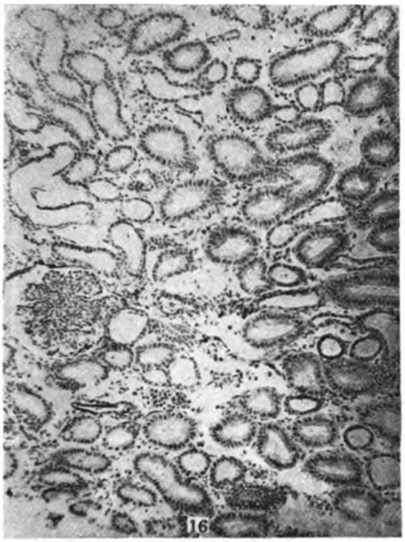
18 Normal excretory tubule.

EFFECT OF TARTARIC ACID

19 Small excretory tubule showing granular degeneration with shrunken opaque nuclei.

20 Extreme stage of granular degeneration. The tubule is represented by a mere mass of débris. Note the surrounding cells with large eosinophilic granules.

21 Hyaline degeneration, showing hyaline droplets and shrunken nuclei.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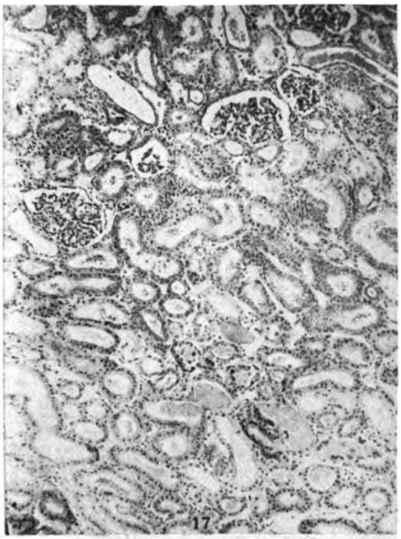
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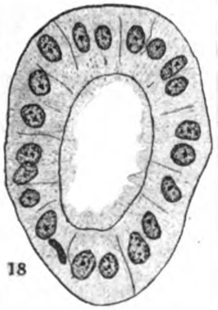
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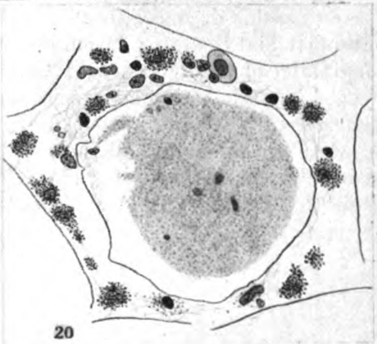
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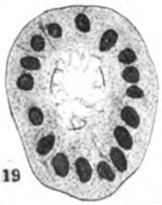
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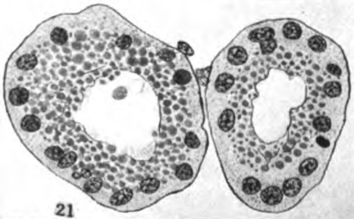
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PLATE 6

EXPLANATION OF FIGURES

KIDNEY, SPLEEN, AND GILL. URANIUM NITRATE

(See figure 18 for normal kidney)

22 Mild granular degeneration.

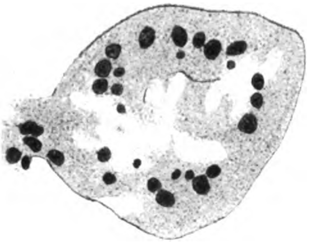
23 Late granular degeneration.

24 Granular and hydropic degeneration, but not typical of the latter type.

25 Beginning of hyaline degeneration. Late stage not shown.

26 Nuclei found in the blood-vessels and connective tissue of kidney, coming probably from endothelium of spleen and gills.

27 Nuclei from the endothelium of the spleen and gill sinuses and probably representing abnormal amitosis in production of phagocytes. The cell 'x' represents a mitotic figure seen in the spleen.



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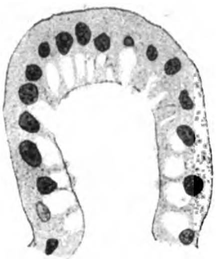

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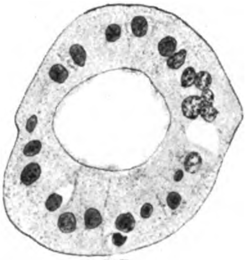

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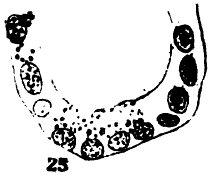

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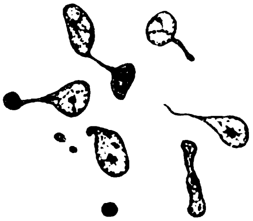

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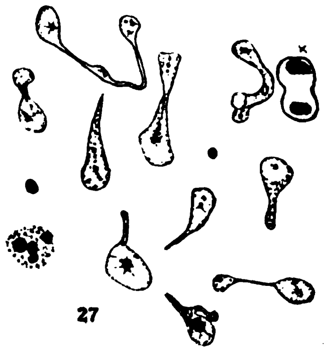

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RICHARD RATHBUN AND HIS CONTRIBUTIONS TO ZOOLOGY.

In the October number of the Journal (page 620) mention was made of the death of Dr. Richard Rathbun, Assistant Secretary of the Smithsonian Institution and for nearly twenty years in charge of the United States National Museum.

Dr. Rathbun states in a brief autobiography which has kindly been placed in the hands of the writer that his interest in science dated from 1868 when, at the age of sixteen years, he was attracted by the fossils in the quarries near Buffalo, New York, in which he was employed as financial clerk and overseer of work. Fascinated by the glimpses of the ancient life of the world as revealed in Hugh Miller's "The Old Red Sandstone," young Rathbun set about the collection and study of the Silurian fossils occurring in the sandstones and limestones at Medina, Albion and Lockport, New York.

In these early years, before he was nineteen years of age, he founded the collection of paleontology in the museum of the Buffalo Society of Natural Sciences, and was appointed curator of that section of the museum. At the age of nineteen Rathbun entered Cornell University and continued his paleontological studies under the direction of Charles Fred Hartt. Here he remained for two years, devoting himself largely to the study of a collection of Devonian and Cretaceous Brachiopods and Lamellibranchs from Brazil. The results of these studies are embodied in Rathbun's earliest scientific papers.

The first of these, on the Devonian Brachiopoda of Ereré, province of Pará, Brazil, was completed at Albany with the assistance of Professor James Hall and published in the Bulletin of the Buffalo Society of Natural Sciences.[1] In this paper occur careful descriptions and illustrations of fifteen new species, while the remaining eight species of the collection are referred to previously described forms from North America. This paper was revised and elaborated four years later[2] to include the results of Rathbun's own collections and in it is incorporated a discussion of the relationships of the Devonian fauna of Brazil and North America.

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[1] References to bibliography are placed at the end of the paper.

The Devonian Trilobites and Mollusks of Ereré, Province of Pará, Brazil, collected by Professor Hartt in 1870-71 are described in a joint paper by Hartt and Rathbun, published in the Annals of the Lyceum of Natural History, New York.[3]

The study of the Cretaceous fossils of the Hartt collection required access to the collections in the Museum of Comparative Zoology of Harvard College, and here Rathbun remained from 1873 to 1875. At the same time he served as assistant in Zoology at the Boston Society of Natural History. These studies resulted in a preliminary report on the Cretaceous Lamellibranchs, including detailed descriptions of twelve new species.[4] During the years 1875 to 1878 Rathbun served as geologist to the Geological Commission of Brazil, where he made a study of the geological formations and the scanty mineral resources of several different provinces. While in Brazil he published a report of these geological studies including an account of his search for coal deposits and an extended survey of the coral reefs which lie along the coast.[5]

Several additional papers recording the results of his work in Brazil were published between 1876 and 1879. In one of these[6] the arrangement and formation of the Brazilian coral reefs and the characteristic life of the different faunal zones are explained with great clearness. The Extinct Coral Reefs at Bahia,[7] and the Coral Reefs of the Island of Itaparica, Bahia, and of Parahyba do Norte[8] are the titles of other articles on the same subject. His geological papers include an interesting description of the Brazilian sandstone reefs and the agencies concerned in their formation[9] and reviews of the current literature on the geology of Brazil.[10]

After Professor Hartt's death from yellow fever early in 1878, Rathbun returned to the United States and prepared two papers describing the life and scientific work of his honored friend and teacher.[11]

Rathbun had already acquired some experience in the investigation of marine life from his connection with the explorations of the U. S. Fish Commission as voluntary scientific assistant during the summers of 1874 and 1875, and on his return from Brazil in 1878 he again joined the Commission as a regularly appointed scientific assistant. His interest was thereby diverted from the paleontological field to that of recent animal life, and from this time on his zoological papers deal exclusively with living forms.

He first followed Alexander Agassiz, with whom he had been associated at Harvard, in the study of Echinoids, and his first paper in this new field comprises a list of eleven species of Echinoids from the coast of Brazil.[12] This was followed by a more detailed account of the geographical distributions of all the species of echinoderms known from that locality[13] with descriptions of species new to science. Other papers on echinoderms include reports upon the echini and stalked crinoids collected by the U. S. F. C. steamer Albatross in the Caribbean Sea and Gulf of Mexico,[14] the species of starfishes of the genus Heliaster represented in the U. S. National Museum,[15] and a catalogue of the collection of recent echini in the U. S. National Museum, with notes on geographical distribution.[16]

Rathbun's connection with the U. S. Fish Commission continued until 1896, during which time he published numerous papers describing the results of dredging expeditions off the eastern and southern coasts of the United States, and some of the new species of various groups of invertebrates secured.

In 1879-1880 he was detailed to New Haven, where, under the direction of Professor Verrill, he prepared many duplicate sets of the various species of marine invertebrates represented in the Fish Commission's extensive collections for distribution to museums and other institutions of learning. At the same time he served as Assistant in Zoology at Yale, but in the following year the work was transferred to Washington, where he was appointed curator of the Department of Marine Invertebrates of the U. S. National Museum. Three large series of these sets were eventually prepared and the lists of species in each published in the Proceedings of the U. S. National Museum.[17]

Under Rathbun's direction collections of marine invertebrates were later prepared by the National Museum for various exhibitions. The catalogue of the collection of economic crustaceans, worms, echinoderms and sponges for the Great International Fisheries Exhibition at London in 1883[18] contains an excellent account of the economic importance of these groups and the industries in which they are concerned, while another catalogue[19] describes the collection illustrating the scientific investigation of the sea and fresh waters. He also prepared and published the records of the dredging stations of the U. S. Fish Commission for many years, in part with the coöperation of Sanderson Smith.[20]

During this period Rathbun continued his systematic studies on various groups of invertebrates, publishing annotated lists of corals in the U. S. National Museum, with diagnoses of a number of new species[21] and a catalogue of the marine fauna of Provincetown, Mass.[22] He was also interested in the parasitic copepods, of which he published a list of the species in the United States National Museum[23] and described many new forms.[24]

In the economic aspects of marine biology Rathbun produced the best of all his zoological work, and rendered a great service both to science and industry. His account of the natural history of crustaceans, worms, radiates and sponges[25] in Goode's Natural History of Aquatic Animals, published in connection with the Tenth Census, is a work of the highest excellence. This was followed by three extensive reports on the history and methods of the fisheries. Of these, the first deals with the crab, crayfish, lobster, shrimp and prawn fisheries,[26] the second with the leech industry and trepang fishery,[27] and the third on the sponge fishery and trade.[28] Other reports published in connection with the Tenth Census include an account of the various fishing grounds of North America,[29] and a survey of the ocean temperatures of the eastern coasts of the United States.[30] Altogether these reports comprise 550 quarto pages and 106 plates, and they form one of the most important of all contributions to marine economic zoology.

Other economic papers include notes on the decrease of lobsters,[31] lobster culture,[32] transplanting of lobsters to the Pacific Coast,[33] the shrimp and prawn fisheries,[34] methods of deep-sea dredging,[35] investigations by the schooner Grampus,[36] a review of the fisheries in the contiguous waters of the State of Washington and British Columbia,[37] and an introduction to the report on the Albatross explorations in Alaska,[38] in addition to yearly contributions to the reports of the U. S. Commission of Fish and Fisheries from 1888-1896 respecting food fishes and the fishing grounds. That he could also write in a popular manner is shown by his articles on the "Giant Squid,"[39] and "The United States Fish Commission."[40]

Rathbun's publications by no means represent his major service to the Fish Commission, for his duties as chief executive officer and in charge of the scientific work of the Commission, in addition to several terms as acting commissioner, gave him the opportunity, for which he was so well fitted, of devising and directing the scientific investigations of the entire staff. To his skillful management much of the practical success of the Commission previous to 1896 is due.

Very important services to economic zoology were rendered by him in preparing the evidence for the case of the United States in the Paris fur seal Tribunal, in arranging for yearly surveys of the fur seal population in the Bering Sea, and later as the United States representative on the "Joint Commission with Great Britain relative to the preservation of the fisheries in waters contiguous to the United States and Canada." During these years (1891-1896) the fisheries conditions were very thoroughly investigated and an extended report published by Congress.[41]

In 1896 Rathbun severed his connection with the Fish Commission and entered upon the administrative service of the Smithsonian Institution, of which he was appointed Assistant Secretary early in 1897. His natural generosity caused him to devote more and more of his time and energies to his executive duties and from 1899, when he was placed in charge of the National Museum, he had little opportunity for original investigations.

His later writings were mainly limited to his administrative reports of the National Museum[42] during the years 1899 to 1917 in which he displayed great skill in the forceful presentation of the details required. To the building up and exhibition of the priceless collections of this great national institution and to the encouragement of its scientific research he gave his entire time and thought for upwards of twenty years.

His last publications relate to the culminating efforts of his life—the great new natural history building of the National Museum[43] and the national gallery of art,[44] together with a paper of historical interest on the history of the Columbian Institute for the Promotion of Arts and Sciences.[45]

On the day of Dr. Rathbun's death, July 16, 1918, the staff of the Smithsonian Institution recorded "their profound sorrow at the loss of a sincere friend, an executive officer of marked ability and one whose administration has had a wide influence upon the scientific institutions of the nation."

To his far-sighted wisdom, administrative ability, and untiring zeal systematic and economic zoology owe much, and to him the American public for generations will be indebted for an exposition of natural history which has few rivals.

For a brief account of the personal side of Dr. Rathbun's life, his success as an executive of the U. S. Fish Commission, Smithsonian Institution and National Museum, together with the honors which were accorded him, the reader is referred to Dr. Marcus Benjamin's recent paper in Science.[46]

Richard Rathbun's scientific career may be summarized in a few words; a youthful enthusiast in paleontology, an investigator of the Devonian and Cretaceous deposits and the coral reefs of Brazil, a contributor to systematic paleontology and zoology; in middle life a leading authority on the economic aspects of marine zoology and the means of its investigation; but most prominently and gratefully recognized in his full maturity for his remarkable ability in the administration of the United States National Museum; to him in large measure the successful development of this great national center of research and exposition is due.

WESLEY R. COE.

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